

***Nysius huttoni* (Hemiptera: Lygaeidae):
life history and some aspects of its
biology and ecology in relation to
wing development and flight**

A thesis submitted in partial fulfilment
of the requirements for the degree
of

Doctor of Philosophy

in

Entomology

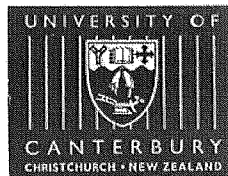
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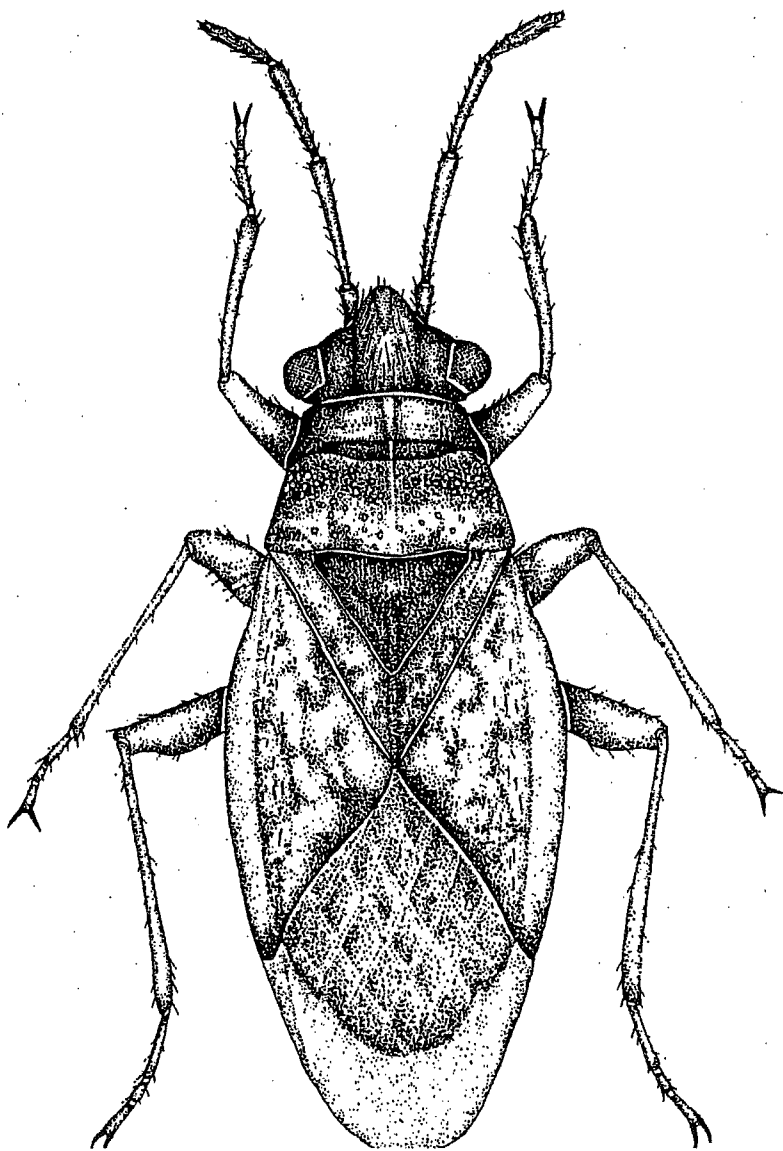
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June 2001

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Nysius huttoni White 1878 (Hemiptera: Lygaeidae)

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Acknowledgements

I extend many thanks to my supervisors, Mr Peter Johns and Professor Mike Winterbourn for their help and guidance throughout my Ph D studies. Extra special thanks go to them for their help with my English and grammar and writing style of my thesis, and for their understanding and patience while reading my thesis drafts over and over again. I thank Dr Raphael Didham for also reading a thesis draft and giving me some good ideas, suggestions and comments. I greatly appreciate the stimulating discussions and useful guidance that Dr Roderick Macfarlane provided during my thesis work. My thanks to Professor Robert Jackson, who kindly kept track of progress on my thesis.

Special thanks also to Dr Hazel Chapman and Mrs Margaret Johns for their assistance with the identification of host plants of *N. huttoni*, and to Cor Vink who helped identify spiders.

Many thanks to Bruce Lingard, Linda Morris and Rosemary Knight for providing experimental equipment and facilities whenever I needed them, and for many other services and help. A special 'thank you' also goes to Victor Mencil for preparing the incubators used to rear insects, and for checking the temperatures and light regimes of the incubators so frequently. Due to his excellent work, I completed the rearing work successfully and obtained reliable results. Thanks to Terry Williams for taking photos of my insects and Jan McKenzie who helped me print them. Also, a big 'thank you' to John Scott for his help with computers and for the special consideration he gave me. Graeme Bull is thanked for assistance with histology and for spending endless hours showing me how to cut sections.

I particularly want to thank Nick Etheridge for the concern he had for me and my family, for the many ways he helped me, and for always having cheerful words that warmed my heart and encouraged me to overcome difficulties and complete my research. Lyn de Groot, Tracey Robinson, and Helen Spinks also provided invaluable and enthusiastic assistance with endless things I needed since I came to the Zoology Department. Their help was greatly appreciated.

Finally, special thanks must go to my friends, Shen Xiao Dong, Jiao Wen Xu, Zhang Yao, and Jiao Xin Qing, for their help in collecting insects in the field, measuring wing lengths of insects, especially in rearing insects in the laboratory when I was away, and for the good ideas, suggestions, comments, and opinions they gave me when we talked about my research. My thanks to Linda Yu, whose assistance with editing the thesis was invaluable.

Financial assistance for this research was provided by a University of Canterbury Doctoral Scholarship and a grant from the Entomological Society of New Zealand. I am very grateful for this support.

Abstract

Aspects of the life history, general biology and wing polymorphism of the Lygaeid bug, *N. huttoni* are examined in this thesis. The effect of temperature on development of *N. huttoni* was studied in the laboratory at 5 constant temperatures (15, 20, 25, 30, and 35°C) and lab temperature (range 12.5-29.5°C, mean 20.3°C). Time required for development from egg to adult ranged from 108 days at 15°C to 17.8 days at 35°C, and varied in a linear manner with temperature. Threshold temperatures and thermal unit requirements were obtained for eggs, each instar, the total nymphal stage, and overall development. Photoperiod also affected the development of *N. huttoni*. At 20°C, development from egg to adult was significantly faster under a 12-h photoperiod (57.7 days) than under 16-h (63.1 days) or 8-h photoperiods (65 days). At 27.5°C, however, the influence of photoperiod was less clear.

In the field, *N. huttoni* had three overlapping generations a year and three peaks in abundance. Developmental duration and period of occurrence of each generation and life stage were recorded. Adults of the third generation overwintered from mid-April - early May, and emerged from hibernation in late August or early September. Three copulation peaks and three oviposition peaks occurred each year. Third-generation adults underwent reproductive diapause from late summer to early spring and the diapause was terminated within about two weeks in early spring. Naturally-induced diapause of adults collected from the field in autumn was also broken within about two weeks at 25°C/12L:12D in the laboratory. Adults entered diapause in about one month under 20°C/8L:16D conditions and terminated diapause in about one week when transferred to 25°C/16L:8D.

Sex ratios of laboratory-reared populations were 1:0.90-1:1.31 (♀:♂) at six experimental temperatures and 1:0.69-1:1.86 under three photoperiods. Mean

annual sex ratios of field-collected insects ranged from 1:1.05 to 1:1.23. Experiments showed that a single copulation could not fertilise a female for life, although sperm remained viable for one to three months. Parthenogenesis was not found. Laboratory experiments on the tolerance of adults to starvation indicated that starvation-longevity of first-generation adults (mean 4.2 days) was about twice that of second- and third-generation adults (mean 2.2 and 2.5 days). Water prolonged longevity (mean 8.4 days), while glucose in concentrations of 2-40% increased longevity substantially (means 27.6-51.6 days).

Three wing-length morphs were identified in both sexes of *N. huttoni*: macropters (M), sub-brachypters (Sb), and brachypters (B). In the field, the M-form was numerically predominant (94.1%). M x M was the predominant mating combination (up to 80.9%), while ♂M x ♀Sb was the second most common combination (13.7%). Temperature and photoperiod affected wing development with low ($\leq 15^{\circ}\text{C}$) and high ($\geq 30^{\circ}\text{C}$) temperatures and a short photoperiod (8-h photophase) tending to favour the production of Sb- and B-forms. Temperature was a major factor inducing flight.

Four species of insects in 4 families and two orders, and five species of spiders belonging to 4 families in one order all attacked *N. huttoni* under laboratory conditions. However, their significance as predators in the field is unknown since no encounters were seen.

CHAPTER ONE

General introduction and literature review

Chapter 1

General introduction and literature review

Nysius Dallas (1852) (Hemiptera: Lygaeidae: Orsillini) is a large cosmopolitan genus that contains over 100 species. Three species in this genus are now recognised in New Zealand: *Nysius huttoni* White 1878, *N. liliputanus* Eyles & Ashlock 1969, and *N. convexus* (Usinger, 1942). *N. huttoni* was described by Buchanan White in 1878 from adult specimens collected by Hutton and Wakefield. It is indigenous to New Zealand and is widely distributed throughout the country from sea level to 1800 m altitude (Eyles & Ashlock 1969). Myers (1926), Usinger (1943), Gurr (1957), and Cumber (1959) stated that *N. huttoni* occurs throughout the North and South Islands of New Zealand. Alfken (1903) and Kirkaldy (1908) recorded it from the Chatham Islands, whilst Woodward (1954) recorded it from Three Kings Island.

1.1 Damage of *N. huttoni* to crops and economic importance

1.1.1 Damage to wheat

N. huttoni is one of the bugs occurring in New Zealand that attacks wheat grains, and as a result "sticky dough" or "sticky gluten" is produced after processing to flour and baking. In 1936 and 1950, there were major outbreaks of bug damage in Otago and North Canterbury, while four more outbreaks occurred in 1962, 1970, 1973 and 1979 (Swallow & Cressey 1987). The bug attacks the wheat in the milk ripe stage by piercing the grain and sucking up plant juices. In the process, a proteolytic enzyme is injected into the grain to facilitate ingestion of the plant juices. The enzyme residue in the damaged grain interferes with the normal behaviour of the gluten when an attempt is made to bake with flour made from damaged wheat (Gurr 1957).

The proteolytic enzyme rapidly breaks down the dough structure and produces a runny, sticky mess that is quite unusable in the baking trade. Even a small amount of bug infestation is sufficient to make the flour useless for baking. The effect of three or four bug-damaged grains in a thousand undamaged grains is substantial (Meredith 1970).

Similar bug-damage occurs in Eastern Europe and the Middle East where the insects involved are *Eurygaster integriceps* and *Aelia rostrata*. The chemical effects of their feeding have been shown to follow the injection of a protease that breaks the molecular chains of the dough protein into shorter pieces. New Zealand bug-damage is not a simple protease action of this kind but instead the enzyme activity brings about chemical reduction of the dough proteins, that is, it splits the association between adjacent molecular chains but does not break the chains. Furthermore, the enzyme cannot be extracted by water and is presumably firmly bound to the protein that it attacks (Meredith 1970).

“Sticky dough” or “slimy gluten” produced during fermentation of certain lines of wheat from Otago was first noticed by the director of the Wheat Research Institute in November 1936. Damage to New Zealand wheat by *N. huttoni* was first documented two years later by Morrison (1938) who carried out intensive surveys of the insect pests of wheat crops in Canterbury and North Otago during the summers of 1936-37 and 1937-38. He collected four species of bugs, *Stenotus binotatus*, *N. huttoni*, *Hudsona anceps* and *Dictyotus caenosus* from wheat crops, and all but the last were used in a series of experiments at Lincoln Agricultural College. From these surveys and experiments, Morrison (1938) drew the preliminary conclusion that *N. huttoni* was one of the species capable of attacking New Zealand wheat, with the result that “slimy gluten” is developed. Morrison’s results were confirmed by Cressey *et al.* (1987) and Every *et al.* (1989). The former carried out cage experiment with three species: *N. huttoni*, *Calocoris norvegicus*, and *Sidnia kinbergi* on plots of “Rongotea” wheat at the watery ripe stage of grain development and showed that *N.*

huttoni attacked the grains, producing 23 injured grains per thousand. Every *et al.* (1989) caged *N. huttoni* on plots of two New Zealand cultivars of wheat, Rongotea and Karamu and carried out the baking test, SDS-sedimentation test and an electrophoretic detection test for bug-damage.

Cressey & McStay (1987) showed that bug damage can cause interruption of the later stages of grain filling; the gluten from the damaged wheats liquified rapidly and bug-damaged wheats exhibited high gluten-softening activity, which was very similar to the pattern of activity seen on Russian wheats damaged by the wheat bugs, *Eurygaster* and *Aelia*.

Damage to wheat by *N. huttoni* does not seem to affect germination (Gurr 1957), and bug-damaged grain is perfectly acceptable for seed purposes (Meredith 1970). However, the work by Every *et al.* (1990) showed that the germination capacity of cultivar Rongotea infested by *N. huttoni* was reduced by 72% at the late flowering stage. Damaged wheat is easily recognised in the hand, since the grains show distinct feeding marks: a pale circular area, predominantly on the cheek of the grain, that usually has a dark puncture mark at its centre (Blair & Morrison 1949; Gurr 1952, 1957).

N. huttoni exhibits a distinct preference for different wheat cultivars. Of the observed and tested New Zealand wheat cultivars (Aotea, Kopara, Hilgendorf, Arawa, Rongotea, Karamu and others), Karamu appears to be the most susceptible to damage. Cage experiments (Every *et al.* 1989) showed that cultivar Karamu had 100% visibly injured kernels and 836 puncture marks per 100 kernels, while these two parameters per 100 kernels for Rongotea were 89% and 246 respectively. Other cultivars had even lower percentages and numbers. The work of Every *et al.* (1989, 1990) also showed that kernel weights of bug-damaged samples were lower than those of sound ones. Thus, kernel weights of New Zealand wheat heavily infested during the milk stage were reduced by 1-6% although their protein content was unaltered.

Germination capacity of some of the infested cultivars was also reduced (Rongotea: 72% and Oroua: 87%).

1.1.2 Damage to other plants

N. huttoni has also been recorded attacking linen flax, various grasses, Nassella tussock, broom, strawberry, subterranean clover, red clover, lucerne, shepherds purse, twin cress, Curnow's curse, rape, chou moellier, turnips and other crucifers, as well as a large variety of weed plants. In total, *N. huttoni* has been recorded to have up to thirty-nine host plants. Myers (1921, 1926) and Eyles (1965a, 1965b) recorded 20 species of host plants, Gurr (1952, 1957) 13 species, Woodward (1954) 5 species and Farrell *et al.* (1993) one species.

N. huttoni does considerable damage to young cruciferous crops. When its population is very high, large numbers of feeding punctures around the neck of the seedlings at ground level can lead to cankerous growth of the tissues. This interferes with sap flow and often results in collapse of the plant (Gurr 1952, 1957). Eyles (1965a) reported damage to five cultivated cruciferous crops (marrow-stem kale (*Brassica oleracea*), rape (*B. napus*), swede (*B. napobrassica*), soft turnip (Redglobe) and hard turnip (Greentop) (both *B. rapa*)) by *N. huttoni*. His work showed that *N. huttoni* caged on seedlings caused distortion and then death of parts or the whole plant, and often abnormal sprouting of several meristems.

In contrast to wheat (which is not the preferred food of *N. huttoni*, and is attacked at the edge of the maturing crop only after weeds around the crop have died off in summer), the whole of a cruciferous crop may be destroyed by *N. huttoni* in the seedling stage. The variety of host plants recorded shows that *N. huttoni* is a very adaptable feeder and when the necessity arises can live on almost any cultivated plant as well as a variety of weeds.

1.2 Biology and ecology of *N. huttoni*

N. huttoni has five nymphal instars and overwinters normally as an adult (Gurr 1952). The population is abundant in hot, dry habitats and prefers situations where direct sunlight strikes the ground (Gurr 1957). Farrell & Stufkens (1993) reported that second-generation bugs underwent reproductive diapause and suggested that it might be induced by the shortening daylength of late summer.

Eyles (1960) redescribed the imago of *N. huttoni*, described the immature stages, and gave illustrations of all stages for the first time. He (Eyles 1963a) reported on fecundity and oviposition rhythms, and described the development of eggs and nymphal instars (Eyles 1963b) of *N. huttoni* reared in a green house at Palmerston North over a complete breeding season.

Eyles (1965b) recorded seasonal movements, habitats in different seasons, and described courtship and copulation of *N. huttoni*. Nevertheless, many aspects of its biology and ecology remain poorly understood.

1.3 Number of generations per season

Reports in the literature vary concerning the number of generations of *N. huttoni*. Myers (1926) opined more than one generation per year. Gurr (1952) reported that in Nelson there are at least two generations in normal years, but three or more generations occur under favourable conditions. However, neither of the above authors provided evidence to support their statements. Eyles (1963b) recorded four generations per season, but his results were obtained by rearing *N. huttoni* from egg to adult in an unheated greenhouse at Palmerston North over a complete breeding season. Based on field observations, Eyles (1965b) reported that in Palmerston North there are at least three and possibly four generations per season in the field. In contrast,

Farrell & Stufkens (1993) reported that two generations of *N. huttoni* developed per season in Canterbury, New Zealand. The conflicting nature of these reports suggests that the life history of *N. huttoni* is highly flexible, and the number of generations may be determined by environmental factors such as temperature. This possibility is one of the aspects considered in detail in this thesis.

1.4 Polymorphism of *N. huttoni*

1.4.1 Wing Polymorphism

N. huttoni appears to be unique amongst the Orsillini in having three wing-forms in both sexes (Eyles 1960). These forms are macropters, in which the wings extend beyond the posterior of the abdomen; sub-brachypters, in which the posterior of the wing is level with, or scarcely exceeds, the posterior of the abdomen; and brachypters, in which the posterior of the wing does not reach the posterior of the abdomen. Eyles (1960) discussed the effect of temperature on wing form and stated that temperature is probably not a major factor affecting wing form in *N. huttoni*. Since then, however, further research on wing form in this species has not been published. A major section of this thesis considers this question.

1.4.2 Body size polymorphism

Body size of both sexes varies in field populations as shown by Eyles (1960) who identified three size groups as follows (dimensions in millimetres):

Group I: Larger individuals-Male: length 3.55-3.86; width 1.32-1.39.

Female: length 3.74-4.34; width 1.61-1.75.

Group II: Medium sized individuals-Male: length 3.00-3.48; width 1.15-1.32.

Female: length 3.36-3.74; width 1.44-1.53.

Group III: Smaller individuals-Male: length 2.38-3.00; width 0.94-1.15.

Female: length 2.47-3.19; width 1.20-1.32.

Eyles (1960) pointed out that the macropterous form has only been observed in Group I. In Groups II and III the three forms, macropterous, sub-brachypterous and brachypterous occur. Thus, there are seven different “kinds” of individuals within the species (based on size and wing-form).

As stated by Blakley (1981), the timing of metamorphosis in insects imposes a limitation on the duration of larval growth, which directly affects adult size, since adults cannot grow. In milkweed bugs, *Oncopeltus cingulifer cingulifer* and *O. fasciatus* (Hemiptera: Lygaeidae) adult ecdysis occurs only after a critical size (indexed by weight) is attained (Blakley & Goodner 1978). Blakley (1981) reported that achievement of this critical body size provides the proximate stimulus for the metamorphic moult in *Oncopeltus* and other hemipterous and holometabolous insects, and that the effects of size-dependent stimuli on metamorphosis vary among different insects. He also found that growth rate of *Oncopeltus* varied when nymphs were kept on individual host plants that differed greatly in nutritional quality. Variation in growth rate resulted in marked variation in adult size. Viewed in an ecological context (Blakley & Goodner 1978), size-dependent regulation of development in *Oncopeltus* represents a life history adaptation to unpredictable conditions for larval growth. Eyles (1960) discussed the variation exhibited in body size in *N. huttoni*, however, the mechanisms determining body size variation and its adaptive significance are as yet unknown.

1.5 Objective and aims of my research

Using *N. huttoni*, the overall objective of my study was to obtain new information on some basic adaptations of insects: life history, wing polymorphism and flight, reproductive diapause, and effects of temperature and photoperiod on development. I chose *N. huttoni* for this study because it

appears to be a serious clover pest in New Zealand. My goal has been to gain new information that may be significant for the management of this important, but poorly understood, species.

Development of insects is strongly related to temperature. In general, insects complete their development within a definite temperature range. Above the lower developmental threshold, development rates increase proportionally as temperature increases, above which development rates and survival decline dramatically (Wagner *et al.* 1984). These relationships are often interpreted as a constant heat accumulation (day degrees) that is required for development. Many thousands of papers have reported the effects of temperature and photoperiod on insects. However, with the exception of limited studies on the basic biology (Eyles 1965b; Gurr 1952, 1957), no published information was found to demonstrate the importance of temperature and photoperiod to development of *N. huttoni*. The objective of present study was to investigate the influence of temperature and photoperiod on development, longevity, and fecundity. This objective included establishing a regression equation between temperature and development and determining the developmental threshold temperature and thermal constant for various stages for *N. huttoni*. The outcome of this study could help provide a means of predicting and forecasting the occurrence of this species in the field and at the same time, a basis for ecologically sound management strategies.

Successful management of *N. huttoni* requires a detailed knowledge of many aspects of its biology and intensive investigation of life history. However, details are lacking on many aspects of the biology and ecology of *N. huttoni* crucial to effective development of management strategies. Information on its development would enable predictions to be made regarding field population development and phenology.

Insects generally encounter great changes in climatic conditions from year to year. To various degrees, insects adapt to these changes. Therefore in my studies, I examined various aspects of the seasonal biology of *N. huttoni* under field conditions. My main objectives were to determine for *N. huttoni* the seasonal occurrence, developmental duration, number of generations completed annually, sex ratio and seasonal variation, seasonal copulation and oviposition patterns, and seasonal variations in population density.

Diapause is an important adaptive mechanism for insect survival under unfavourable conditions. Although it has not been demonstrated experimentally in *N. huttoni*, it has evolved in many other hemipteran species (eg., *Pyrrhocoris apterus*, Kalushkov *et al.* 2001; *Orius insidiosus*, Ruberson *et al.* 1991), and members of Lygaeidae (Dingle 1974, Solbreck 1979). Therefore, it is reasonable to hypothesize that phenology of *N. huttoni* includes a reproductive diapause. My first objective related to diapause was to document the existence of diapause in this species by field observations and dissection of female adults collected from the field. My second objective was to determine the effect of photoperiod on reproductive diapause under laboratory conditions, that is, to test the hypothesis that short-day photoperiod induces an adult diapause and long-day photoperiod promotes reproduction.

Wing polymorphism is a common phenomenon in many insects including species of Lygaeidae (Tanaka & Wolda 1987, Cherry 2001). *N. huttoni*, which is widely distributed in New Zealand, is a ground-dwelling polyphagous species (Eyles & Ashlock 1969). Both sexes of this species show wing polymorphism (Eyles 1960). However, there are few quantitative field data on the occurrence of these wing forms in *N. huttoni*. Although some environmental factors such as temperature, photoperiod, food quality and population density have been reported to be responsible for the determination of wing forms in many species (Harrison 1980), little attention has been devoted to the factors affecting wing development in *N. huttoni*. On the other

hand, the relationships between environmental factors and flight have been examined in many other species, but not in *N. huttoni*. The objectives of this study were to determine the seasonal occurrence of wing polymorphism in *N. huttoni* and to determine if wing polymorphism is correlated with the photoperiod. Also, flight of *N. huttoni* and affecting factors were investigated.

“Based on the many detailed studies of natural enemies of major insect pests in their native homes, it would seem that most plant-feeding insects have more than, and generally several to many, natural enemies” (Debach & Rosen 1991). Until recently, however, knowledge of the natural enemies of *N. huttoni* was especially lacking. The initial survey of natural enemies and primary feeding test are summarised in my study. This information is necessary for determining the potential for biological control of *N. huttoni*.

The specific aims of my research on *N. huttoni* were to:

1. Investigate the habitats, host plants, and general habits of the species.
2. Investigate the life history in the field and controlled laboratory conditions, in order to determine the seasonal occurrence and number of generations per season, and other aspects of seasonal biology and ecology.
3. Determine the effect of temperature and photoperiod on development.
4. Ascertain the reason for wing polymorphism experimentally, observe and investigate flight activity and the environmental factors influencing flight.
5. Test tolerance of adults to starvation and the effects of carbohydrate and water alone on adult longevity.
6. Verify whether *N. huttoni* undergoes a reproductive diapause, and if so,

determine the factors inducing and terminating diapause.

7. Search for possible control organisms that might reduce *Nysius* populations in a managed situation.

My research combined extensive field research with intensive laboratory rearing of insects in controlled chambers. A piece of wasteland in Hornby, south of Christchurch was used as the main field research site where sampling and observations were made from 1995 to 1999. The site is about one hectare and is covered with variety of wild plants dominated by grasses, but with many of the host plants listed in Chapter 2 Table 2-2.

CHAPTER TWO

Habitats and host plants of *N. huttoni*

Chapter 2

Habitats and host plants of *N. huttoni*.

2.1 Introduction

In natural environments, organisms live together. During their growth, development and reproduction, very complex interactions are often formed between individuals of the same species (intraspecific) or between those of different species (interspecific). In number of species, insects are the largest group in animal kingdom. Many of them are herbivorous, while others are carnivorous and parasitic (Yazdani & Agarwal 1997). Food is one of the important biotic factors in insect environments. The insects have undergone a long period of being subject to processes of coevolution and coadaptation with their host plants. Different insect species have different host-plant ranges. Even the various stages of the same species may have different host plant ranges. Thus, different insects have developed different patterns of associations with their host plants (Hodkinson & Hughes 1982).

The quantity and quality of food have great influence on survival, longevity, distribution, reproduction and speed of development of insects. The quality of available food plays an especially important role in egg production, larval development and size of insects (Yazdani & Agarwal 1997). Different plant species has different colour, odour, taste and form. Any herbivorous insect has to use these basic sensory cues to locate and recognise its suitable host plant(s) from its habitat and ensure that its period of feeding activity is coincident with the periods of plant availability (Hodkinson & Hughes 1982). Also, the ability of an insect to find suitable host plants depends on the insect's mobility and pattern of dispersal (May & Ahmad 1983). Polyphagous insects are flexible in choice of host plants, depending on the changing quality of plants and their environment (Brodbeck *et al.* 1990). "Each plant species is most abundant at a particular successional age" (Leps *et al* 2001). The relative

importance of each host in supporting insect population varies with the season.

Hemipteran mouthparts are different from those of other insect orders. Their elongate mouthparts are adapted for piercing and sucking both plant and animal material (Gullan & Cranston 2000). Many species of Hemiptera are serious agricultural and horticultural pests. The majority of hemipteran species present on arable land are species more commonly associated with grassland or ruderal habitats. The number of hemipteran species present on a site is influenced by the species diversity (number and abundance of species) of the plant community present (Brown 1991). Plant-feeding (phytophagous) species are known to respond to variation in both vegetation structure and plant nutritional quality (Prestidge 1982, Prestige & McNeill 1983, Denno & Roderick 1991, Sanderson *et al.* 1995).

Since the diversity of hemipteran communities is strongly linked to plant species and structural diversity, assessment of these communities may provide a good indication of the potential of the vegetation for supporting phytophagous insects and, in the case of the Heteroptera, of the suitability of the habitat for supporting predator and detritivore species (Denno & Roderick 1991).

Among herbivorous insects, some are monophagous species that feed on one plant taxon; while others are oligophagous species that feed on few plant taxon; still others are polyphagous species that can feed on many plant groups (Hodkinson & Hughes 1982, Gullan & Cranston 2000). *N. huttoni* is a polyphagous insect pest that feeds on various cultivated and wild plants (Eyles 1965b). Further survey of host plants and habitats is important for a comprehensive understanding of the biology and ecology, as well as management of, *N. huttoni*. General notes on habitat and its limits of *N. huttoni* have been given by Gurr (1957) and Eyles (1965b). *N. huttoni* has been

recorded attacking 40 species of host plants (Table 2-1). The present study was undertaken to obtain more information on its habitats and host plants.

Table 2-1. Host plants of *Nysius huttoni* noted by previous authors.

No.	Scientific name	Common name	Reference
1	-----	grasses	Gurr 1952, 1957
2	<i>Agrostis tenuis</i> Sibth	brown top	Myers 1921, 1926; Eyles 1965a, 1965b
3	<i>Anagallis arvensis</i> L.	scarlet pimpernel	Myers 1921, 1926; Eyles 1965a, 1965b
4	<i>Brassica napobrassica</i> Mill.	swede	Myers 1921, 1926; Eyles 1965a, 1965b
5	<i>Brassica napus</i> L.	rape	Gurr 1952, Eyles 1965a
6	<i>Brassica oleracea</i> L.	choumoellier	Gurr 1952, Eyles 1965a
7	<i>Brassica rapa</i> L.	turnips (soft and hard)	Gurr 1952, Eyles 1965a
8	<i>Brassica</i> spp	cruciferous crops	Gurr 1952, 1957
9	<i>Calandrinia caulescens</i>	Curnow's curse	Gurr 1952, 1957
10	<i>Capsella bursa-pastoris</i> (L.) Medik.	shepherd's purse	Gurr 1952, 1957; Farrell & Stufkens 1993
11	<i>Cassinia leptophylla</i> R. Br.	tauhinu	Myers 1921, 1926; Eyles 1965a, 1965b
12	<i>Chenopodium triandrum</i> Forst	-----	Woodward 1954
13	<i>Coronopus didymus</i> L. Sm.	twin cress	Gurr 1952, 1957; Farrell & Stufkens 1993
14	<i>Disphyma australe</i> (A. Cunn.) Black	-----	Woodward 1954
15	<i>Fragaria</i> sp	strawberry	Gurr 1952, 1957
16	<i>Holcus lanatus</i> L.	Yorkshire fog	Myers 1921, 1926; Eyles 1965a, 1965b
17	<i>Hypochaeris radicata</i> L.	cats ear	Myers 1921, 1926; Eyles 1965a, 1965b
18	<i>Juncus bufonius</i> L.	toad rush	Myers 1921, 1926; Eyles 1965a, 1965b
19	<i>Leptospermum ericoides</i> A. Rich	flowering kanuka	Woodward 1954
20	<i>Linum marginale</i> A. Cunn	linen flax	Gurr 1952, 1957
21	<i>Linum</i> sp.	-----	Myers 1921, 1926; Eyles 1965a, 1965b
22	<i>Lolium perenne</i> L.	perennial ryegrass	Myers 1921, 1926; Eyles 1965a, 1965b
23	<i>Medicago sativa</i> L.	lucerne	Myers 1921, 1926; Usinger 1942; Gurr 1952
24	<i>Meryta sinclairii</i> (Hook.) Sum.	puka	Woodward 1954
25	<i>Myoporum laetum</i> Forst f	prostrate ngaio	Woodward 1954
26	<i>Nassella trichotoma</i>	Nassella tussock	Gurr 1952, 1957
27	<i>Paspalum dilatatum</i> Poir	paspalum	Myers 1921, 1926; Eyles 1965a, 1965b
28	<i>Polygonum aviculare</i> L.	wireweed	Eyles 1965b, Farrel & Stufken 1993
29	<i>Rumex acetosella</i> agg L.	sheep sorrel	Myers 1921, 1926; Eyles 1965a, 1965b
30	<i>Sarothamnus scoparius</i> L.	broom	Gurr 1952, 1957
31	<i>Silene gallica</i> L.	catch fly	Myers 1921, 1926; Eyles 1965a, 1965b
32	<i>Soliva sessilis</i> Ruiz & Pav	Onhunga weed	Myers 1921, 1926; Eyles 1965a, 1965b
33	<i>Spergularia campestris</i>	sand spurrey	Myers 1921, 1926; Eyles 1965a, 1965b
34	<i>Stellaria media</i> (L.) Vill	chickweed	Farrell & Stufkens 1993
35	<i>Trifolium dubium</i> Sibth	suckling clover	Myers 1921, 1926; Eyles 1965a, 1965b
36	<i>Trifolium pratense</i>	red clover	Myers 1921, 1926; Eyles 1965a, 1965b
37	<i>Trifolium repens</i> L.	white clover	Myers 1921, 1926; Eyles 1965a, 1965b
38	<i>Trifolium subterranean</i> L.	subterranean clover	Gurr 1952, 1957
39	<i>Triquetrella papillata</i>	moss	Myers 1921, 1926; Eyles 1965a, 1965b
40	<i>Triticum aestivum</i> L.	wheat	Morrison 1938, Gurr 1957

Housing facilities provided by the host-plant are important because the host plant does not only provide sustenance, but also provides shelter (Schoonhoven *et al.* 1998). In my research, plants that were found to support development of *N. huttoni* were determined as feeding hosts (food). Others were listed as host plants. Although their importance as food was uncertain, they were found to be infested with *N. huttoni* in presumed shelter sites. Thus, further feeding tests are needed to determine the importance of each plant and the preference of *N. huttoni* for it as food source.

2.2 Materials and methods

A survey of the habitats of *N. huttoni* was undertaken to reaffirm previous work and record new associations with wild and cultivated plants. The survey was carried out in the vicinity of Christchurch from July 1995 to July 1999.

Collections of the host plants for *N. huttoni* were made and identified in the laboratory throughout the seasons from July 1995 to July 1999. Sites surveyed were:

- (1) Research site (see Chapter 1).
- (2) Small specific habitats where *N. huttoni* occurred on a single host plant (e.g., wall bases of a house where many adults and nymphs of *N. huttoni* were found under cultivated flowers). The observations were made at 10-day intervals to determine whether or not the *N. huttoni* population would live under a single host plant throughout the year.
- (3) A wheat field chosen to observe whether *N. huttoni* fed on wheat from September 1995 to January 1996, and whether *N. huttoni* attacks wheat in both seedling stage and ripe stage. The wheat field chosen for observation was close to a road and the strip of grassed, waste piece land between the

field and road had large numbers of *N. huttoni* present. Observations were made weekly.

(4) Apple orchards were investigated regularly to find *N. huttoni*. Each time both the ground and the fruit trees were checked.

(5) Other likely habitats.

2.3 Results and discussion

2.3.1 Habitats

N. huttoni lives on dry, hot ground with sparse herbal vegetation. It was found in waste land, pastures, gardens, lawns, shingle and sandy riverbeds, under flowers belonging to the Compositae at the bases of sunny house walls, in gravel car parks, on bare ground surfaces among grass on the campus of University of Canterbury, and on stony, sandy ground supporting a few weeds, beside roads. During the summer, *N. huttoni* can be found almost anywhere where weeds are available and vegetation is sparse enough for full sunlight to reach the bare ground.

N. huttoni was seldom found where the vegetation was so dense that it precluded movement of the insects. It was not found in damp habitats beside a river and pool where the ground was damp even where it was exposed and the vegetation was sparse.

In orchards, *N. huttoni* were found only on some of the bare ground between lines of fruit trees, but numbers were very low and sometimes only a few individuals were found. These low numbers in the orchards were possibly due to the denser vegetation on the ground, dampness due to regular irrigation, pesticides used to control weeds, other insect pests and tree diseases, or

because little sunlight reached the ground through the branches. No *N. huttoni* were found on fruit trees at any time.

These features indicates that *N. huttoni* does not attack fruit trees and that the orchard was not a suitable habitat.

2.3.2 Host plants

Forty-two species of host plants were found in the survey in Christchurch, and are listed in Table 2-2.

Shepherd's purse, twin cress, broom (Gurr 1952, 1957; Farrell & Stufkens 1993), wireweed, Yorkshire fog, cats ear, perennial rye grass, sheep sorrel, sand spurrey and white clover (Eyles 1965b) have been recorded before, but other host plants are recorded for the first time.

At the research site, observations showed that sand spurrey *Spergularia rubra* and *Polygonum aviculare* L. were the favorite foods of *N. huttoni*. In spring and summer, *N. huttoni* aggregated under these two plants in much larger numbers than under other host plants. Also, *Spergularia rubra* was the overwintering host. In very hot summer seasons when all of the plants in the habitats dried and died, the population of *N. huttoni* was reduced greatly and the insects stayed under *Spergularia rubra*.

In the field, *N. huttoni* seemed to exhibit a preference for some specific plants. Thus, nymphs and adults were both found under or around creepers. Small populations occurred around or under plants that grew vertically. For example, *Polygonum aviculare* L. was one of the typical creeping plants under which very large populations gathered during spring and summer. Under or around *Spergularia rubra*, *N. huttoni* was observed to gather in large populations year round. In contrast, low numbers of nymphs and adults were found under or

around more upright plants such as *Malva parviflora* L., *Erodium cicutarium*, and *Lapidium pseudotasmanicum* Thell. The creeping plants, apart from being used as foods, were used as shelters when the weather was very hot.

Table 2-2. Host plants of *N. huttoni* in the Christchurch area survey 1995-2000.

No.	Scientific name	Common name	Importance of host
1	<i>Capsella bursa-pastoris</i> (L.) Medic.	Shepherd's purse	Food
2	<i>Cirsium vulgare</i> (Savi) Ten.*	Scotch thistle	
3	<i>Coronopus didymus</i> (L.) Smith	Twin cress	
4	<i>Crepis</i> sp.*	Hawksbeard	Food
5	<i>Echium vulgare</i> L.*	Viper's bugloss	
6	<i>Elytrigera</i> sp.*	-----	
7	<i>Erigeron karvinskianus</i> DC.*	Mexican daisy	Food
8	<i>Erodium cicutarium</i> (L.) L'Her.*	Storksbill	
9	<i>Erodium</i> sp.*	Cranesbill	
10	<i>Erodium</i> sp.*	-----	
11	<i>Geranium</i> sp.*	Cranesbill	
12	<i>Holcus lanatus</i> L.	Yorkshire fog	
13	<i>Hordeum marinum</i> Huds.*	Barley grass	
14	<i>Hypochoeris radicata</i> L.	Catsear	Food
15	<i>Lapidium pseudotasmanicum</i> Thell.*	Narrow-leaved cress	Food
16	<i>Lobularia maritima</i> (L.) Desv.*	Alyssum	Food
17	<i>Lolium perenne</i> L.	Perennial ryegrass	
18	<i>Malva parviflora</i> L.*	Small-flowered mallow	Shelter
19	<i>Malva sylvestris</i> L.*	Large-flowered mallow	
20	<i>Marrubium vulgare</i> L.*	White horehound	
21	<i>Poa annua</i> L.*	Annual poa	Food
22	<i>Polygonum aviculare</i> L.	Wireweed	Food
23	<i>Pulicaria dysenterica</i> (L.) Bernh.*	Fleabane	
24	<i>Rumex acetosella</i> L.	Sheep's sorrel	
25	<i>Rumex obtusifolius</i> L.*	Broad-leaved dock	
26	<i>Sarothamnus scoparius</i> L.	Broom	Food
27	<i>Spergularia rubra</i> (L.) J.Presl & C.Presl	Sand spurrey	Food
28	<i>Taraxacum officinale</i> G.Weber *	Dandelion	Food
29	<i>Trifolium arvense</i> L.*	Haresfoot trefoil	
30	<i>Trifolium fragiferum</i> L.*	Strawberry clover	Food
31	<i>Trifolium glomeratum</i> L.*	Clustered clover	
32	<i>Trifolium repens</i> L.	White clover	
33	<i>Trifolium subterraneum</i> L.	Subclover	Food
34	Unknown species *	Unknown species	
35	<i>Verbascum thapsus</i> L.*	Flannel leaf (woolly mullein)	
36	<i>Vulpia megalura</i> Rydb.*	Vulpia hairgrass	

* New host plant recorded for the first time. Scientific and common names follow Parsons et al. 1995. Seven unknown species were also noted.

Mexican daisy and alyssum were collected from the sunlit wall bases of several houses. *N. huttoni* bred in summer in large numbers and overwintered

under these two plants. As there were no other plants at the wall bases (cleared of weeds regularly by the house dwellers), there is no doubt that these two hosts are used as food and shelter in summer and as hosts for overwintering by *N. huttoni*. These observations indicate that *N. huttoni* can breed in the same habitat on a single cultivated plant all year around.

No *N. huttoni* were found on wheat from seedling stage to ripe stage between September 1995 and January 1996, although there were plenty of *N. huttoni* on the waste piece of land beside the wheat field. First generation adults were present 10 November 1995 – 10 January 1996 and wheat was in the milk-ripe stage from 10 December to 25 December 1995. Although present, no adults were found to attack wheat kernels. This suggests that wheat is not a normal or preferred food, and that *N. huttoni* is forced to resort to wheat for food when the usual host plants dry off and die as the season progresses. This corroborates the work of Morrison (1938) and Gurr (1957).

Morrison (1938) stated that the reason why wheat bugs attack wheat in one area and not in another is still obscure, and reported that the reason why damage is more prone to occur in certain wheat areas than others might be because of the prevalence of certain weeds, climatic conditions, or a combination of the two factors. Wheat fields do not provide habitats for *N. huttoni* and adults have to fly to wheat fields to attack wheat when it is ripe. Attack on wheat by *N. huttoni* is closely linked with adult flight activity, and flight activity is related to high temperature (see Chapter 7).

Although *N. huttoni* was not found to attack wheat in the wheat field examined in 1995, it may occur on wheat elsewhere, or in other years.

In addition to orchard trees, other trees growing near the research site, beside roads, and elsewhere were checked in spring and summer, but no *N. huttoni* were found on them. Most of the host plants of *N. huttoni* recorded by earlier workers were herbs; only a few of them (*Myoporum laetum* Forst f, *Meryta sinclairii* (Hook.) Sun., and *Leptospermum ericoides*) (Woodward 1954) are woody plants. Therefore, it seems reasonable to conclude that host plants of *N. huttoni* are mainly confined to a range of herbs. This is probably associated with the fact that *N. huttoni* lives just on or close to the ground.

It should be noted that *N. huttoni* is a native species, which, besides occurring on numerous wild plants, also feeds on a wide variety of cultivated plants. The variety of host plants recorded shows it is a very adaptable feeder in New Zealand.

CHAPTER THREE

**Effect of temperature and
photoperiod on development
of *N. huttoni***

Chapter 3

Effect of temperature and photoperiod on development of *N. huttoni*

3.1 Introduction

Both temperature and photoperiod have a strong influence on insects (Beck 1968). They may affect many aspects of biology and morphology of different insect species. They may influence duration of development, female fecundity, adult longevity, and peculiarities of behaviour, and bring about various types of diapause, bisexual or parthenogenetic reproduction, as well as morphological changes ranging from small to large (Zaslavski 1988). Consequently, the occurrence of insects and their population dynamics in the field are controlled by ambient temperature or regulated by photoperiod. For this reason, there has been considerable interest in the relationships between temperature, photoperiod and development of many species of insect in order to be able to predict outbreak times and pest dynamics (Ratte 1985).

The developmental process of insects has the characteristics of a complex chemical reaction (Ratte 1985). That is, the influences of temperature on development of insects reflect the general principle of temperature effect on the rate of chemical processes (Zaslavski 1988). Insects are poikilothermic animals and their body temperature varies more or less with the changes of environmental temperature (Yazdani & Agarwal 1997, Gullan & Cranston 2000). The temperature range for survival of insects is much wider than the range for normal activity and nonarrested development. The latter is often called the favorable temperature for development (Ratte 1985). Over a range of favorable temperatures, the normal physiological activities of insects takes place, but at high and low temperatures, development is retarded and death may occur (Yazdani & Agarwal 1997). Insects, when reared under a

fluctuating temperature, show different developmental times from that when reared under the corresponding constant temperature (Ratte 1985). Under fluctuating environmental conditions, population growth may be faster or slower than under the constant temperature.

An understanding of the phenology of an insect species and the way it is influenced by temperature is important for predicting its seasonal occurrence in integrated pest management (IPM) systems (Yazdani & Agarwal 1997, Gullan & Cranston 2000). Temperature exerts strong effect on insects in a two-fold manner: (1) by direct effects on development and survival and (2) by indirect effects through humidity, food, rainfall and other factors (Yazdani & Agarwal 1997). Gurr (1957) reared *N. huttoni* in an open insectary at ambient temperature and humidity, and recorded the development of 37 eggs and 48 nymphs, and the fecundity of 9 females. Eyles (1963a, 1963b) reported female fecundity of *N. huttoni*, including some observations on oviposition rhythms, and described the development of eggs and nymphal instars reared in an unheated greenhouse with environmental conditions as near as possible to those existing in the field.

However, detailed developmental studies under controlled temperature and photoperiod conditions are lacking for *N. huttoni*. The present study, which deals with the effects of constant temperatures and photoperiods on the development, adult longevity and fecundity of *N. huttoni*, is the first step in a wider research program on the biology and ecology of this species.

3.2 Materials and methods

Shepherd's purse as food for rearing *N. huttoni*. Shepherd's purse was used as food in the rearing experiments because it is an excellent host plant for *N. huttoni* and was available year around. When it was used to feed *N. huttoni*, just the sprig on the upper part of the plant (other than the leaves at

the base) was used. If the leaves at the base of the plant, or those of other species, were used as food for *N. huttoni*, the leaves would become dry and curled at higher temperatures (30 and 35°C), and nymphs, especially first or second instars, would conceal themselves inside the curl. Each time the food was changed, they were not found easily or they could be damaged easily. Furthermore, it was very hard to check whether or not a moult had occurred within a curled leaf. In contrast, the triangular leaves of shepherd's purse do not become curled even if they become dry at higher temperature. On all counts, shepherd's purse was the ideal food for rearing *N. huttoni*.

Temperature and photoperiod used in rearing. The effect of temperature on development was studied at one photoperiod (12-h photophase), 5 constant temperatures (15, 20, 25, 30, and 35 ± 0.5°C) and one fluctuating or lab temperature (mean 20.3°C, range 12.5-29.5°C). The lab temperature is referred to by its average temperature. The effect of photoperiod on development was studied at two constant temperatures (20 and 27.5°C ± 0.5°C) and three photoperiods (16-h, 12-h, and 8-h photophases).

All specimens were held in incubators (74 x 42 x 67 cm). The temperature was controlled by thermostat. Two 18 w daylight spectrum fluorescent tubes were installed in the roof of each incubator to give illumination of 800-1000 lux. A timer was used to regulate the photophase. Each incubator had one temperature and photoperiod treatment. All rearing experiments were conducted from November 1995 - August 1997. The experimental insects were collected from the field at different time according to the requirements for rearing at different temperatures. Temperatures and photoperiods used for rearing, time of collection of the experimental insects, and rearing time of nymphs and adults are summarised in Table 3-1.

By the requirement of the statistical parameters of the experiment, all rearings for egg and nymphal development and adult longevity at different temperature

Table 3-1. Experimental design and treatments for rearing *N. huttoni* at different temperatures and photoperiods.

Temperature (°C)	Photoperiod (L:D)	Time of collection of experimental insects	Rearing time for life history	Rearing time for fecundity and adult longevity
15	12:12	15/01/96	Feb – May 1996	Jan 1996 – Jan 1997
20	12:12	19/11/96	Nov 1996 – March 1997	Nov 1996 – Aug 1997
25	12:12	22/11/95	Nov 1995 – Jan 1996	-----
		16/01/96	-----	Jan – Jul 1996
30	12:12	30/04/96	-----	May – Jun 1996
		10/02/96	Feb – March 1996	-----
35	12:12	13/11/97	Nov – Dec 1997	Nov – Dec 1997
L-T*	Changeable	Emerged from nymphs reared in lab	Nov 1995 – Jan 1996	Jan – Dec 1996
20	16:8	19/11/96	Nov 1996 – March 1997	Nov 1996 – Aug 1997
	12:12	19/11/96	Nov 1996 – March 1997	Nov 1996 – Aug 1997
	8:16	19/11/96	Nov 1996 – March 1997	Nov 1996 – Aug 1997
27.5	16:8	22/11/96	Nov 1996 – Jan 1997	-----
	12:12	22/11/96	Nov 1996 – Jan 1997	Nov 1996 – March 1997
	8:16	22/11/96	Nov 1996 – Jan 1997	-----

* L-T: Lab temperature (mean 20.3°C, range 12.5–29.5°C).

and photoperiod should have been conducted at the same time. However, due to insufficient incubator space provided by the Department and labour (time) requirements, they were carried out in different years. This is a flaw in this experiment. Only the simpler statistical procedures, which are not so sensitive to this flaw, were used.

Adult longevity and female fecundity. Adults were reared from fifth instar nymphs collected from the field in Hornby near Christchurch. After emergence as adults, 35 male/female pairs were set up at each temperature and kept in 15 x 2 cm glass tubes. One pair of adults was placed in each tube. Each tube was covered at one end with muslin cloth fixed by a rubber band so that air could get in, and stoppered at the other end with a plug of cotton wool on which females laid eggs. The tubes were placed in a plate and kept in incubators with different constant temperatures and photoperiods.

At a fixed time each day, the food was renewed, the plug of cotton wool was checked, and number of eggs was recorded. The used plug of cotton wool on which eggs were laid was discarded and replaced by a new one each time. Length of pre-and post-oviposition periods, mating activities, and adult longevity were recorded as well. A trial ended when the last adult died.

Egg development. To determine the duration of the egg incubation period, eggs laid by each female adult at a particular temperature were transferred within 12 hours to a small plastic cylindrical container (5 cm high by 4 cm diameter) together with the cotton wool plug. Each container held the eggs laid by one female. A small hole 0.5 cm in diameter in the lid was covered with fine-mesh metal screen so the container was ventilated but newly hatched nymphs could not escape. Because of difficulty in obtaining sufficient numbers of eggs at 15°C, eggs for the 15°C experiment were oviposited by adults held

at 30°C and then the eggs were held at 15°C for development. All other eggs were incubated at the temperatures at which they were laid.

In the field, *N. huttoni* lays eggs in the soil and eggs can obtain sufficient moisture from the soil for development. In order to provide moisture in each lab containers, a plug of cotton wool was introduced, and a small amount of water was added to it to provide sufficient humidity for egg development. The containers were kept in incubators for hatching and egg development and examined daily. Egg periods (duration from oviposition to incubation) and hatching percentages were recorded.

Nymphal development. To study nymphal development, newly hatched first instar nymphs of *N. huttoni* were transferred to small glass vials (2 cm diameter, 5.5 cm height) on the same day that the eggs hatched. In order to obtain detailed data on development of each instar, nymphs were reared singly; i.e., each vial contained only one nymph. All vials were numbered according to the parent adults and the number of their offspring. The lids of vials were screwed down loosely to allow for aeration. Vials were held in incubators at the same temperatures as the eggs.

At a fixed time each day, food was changed and nymphs were checked as well. Duration of each instar was determined by recording the presence of exuviae, which occurred on the sprigs of shepherd's purse or at the bottom of the vials. Moulded exuviae from first and second instar nymphs were very small and not easy to find, so the sprigs of shepherd's purse and the vials had to be checked carefully each time the food was renewed so that no moult was missed. Mortality of nymphs at each temperature was recorded.

The vials had to be washed and dried thoroughly before they were used to house newly hatched nymphs so that exuviae could be recognised easily from the outside. It was important that fresh and clean sprigs of shepherd's purse

without any water were used as food since nymphs, especially first and second instars, could get stuck in the water and die.

As *N. huttoni* prefers hot, dry conditions in the field, no moisture was supplied to adults and nymphs. Relative humidity was 65-75% within the incubators.

Statistical methods. The regression equation

$$y = a + bx$$

was used to express (1): rate of development where y is the reciprocal of the mean number of days for a stage; (2): mean adult longevity where y is the adult longevity in days, and x is the temperature (°C) in (1) and (2). The equation

$$C = \frac{\sum V^2 \sum T - \sum V \sum VT}{n \sum V^2 - (\sum V)^2}$$

was used to calculate threshold temperature for development (C), and the equation

$$K = \frac{n \sum VT - \sum V \sum T}{n \sum V^2 - (\sum V)^2}$$

was used for determining the thermal constants (K) which express day-degrees required for development of each stage, where n is the number of temperature treatments, T is temperature (°C) and V is the rate of development for each stage at each temperature. Duration of development at each temperature was compared between males and females.

One-way ANOVA was used to compare differences among three and more means and a t-test was used to compare differences between two means.

3.3 Results and discussion

3.3.1 Hatching of eggs

Effect of temperature on hatching percentage of eggs. Hatching success of eggs of *N. huttoni* was high at all constant and lab temperatures tested and

ranged from 79.0% at 15°C to 91.2% at 20°C (Table 3-2). No significant differences in hatching success were found among any of the constant temperature treatments ($P > 0.05$, One-way ANOVA).

Table 3-2. Hatching percentage of eggs of *N. huttoni* at different temperatures.

Temperature (°C)	No. eggs tested	No. eggs hatched	Hatching percentage (% \pm S.E.)
15	169	136	79.0 \pm 4.1 b
20	148	138	91.2 \pm 3.1 ab
25	121	107	89.7 \pm 4.1 ab
30	229	194	84.8 \pm 2.7 b
35	313	242	80.4 \pm 3.5 b
L-T	252	240	95.5 \pm 1.7 a

1. One-way ANOVA was carried out in comparison among hatching percentages at constant temperatures. T-test was carried out to compare hatching percentage at L-T and that at each constant temperature respectively.

2. Numbers followed by the same letter are not significantly different at the 5% significance level.

In the present study, no experiments were undertaken at temperatures below 15°C or above 35°C. However, Eyles (1963b) calculated the hatching percentages of eggs of *N. huttoni* at temperatures below 15°C and above 35°C, and at 45.2°C he obtained a hatching percentage of 90%. He stated that 45.2°C was not the upper limit of temperature tolerance for the eggs. He also placed freshly laid eggs at 3°C for periods of one and two months, respectively, and at 6°C for two months. None of the eggs held at 3°C and 6°C for two months hatched, while 58.5% eggs which were held at 3°C for one month hatched in 16 days after transfer to a greenhouse where the maximum temperatures during October, December and January were 34.4, 27.8, and 31.7°C, respectively, and the minimum temperature ranged from 7.2 to 15.6°C. Apparently, the lower temperature increased incubation time and reduced the hatching percentage of the eggs. In the present study, hatching

percentage was lowest (79%) at the lowest temperature used (15°C) but was not significantly different from that at higher temperatures. Kehat & Wyndham (1972a) recorded that the percentage of eggs of *Nysius vinitor* hatching between 20-35°C was 80-100%, at 15°C it was only 9-18%, and at 12 or 40°C no eggs hatched. Therefore, the data from Eyles's (1963b) work and my work show that *N. huttoni* survives a wider temperature range during embryonic development than *N. vinitor*.

Dunbar & Bacon (1972) examined the hatching success of eggs of three Lygaeidae species at different constant temperatures. They reported that viability of eggs was high in *Geocoris atricolor* (77.1-89.9%) and *G. pallens* (72.0-84.8%) in the temperature range 23.9-35.0°C, and in *G. punctipes* (65.7-88.3%) between 23.9 and 32.2°C. Egwuatu (1977) reported that the hatching percentage of eggs of *Acanthomia tomentosicollis* (Hemiptera: Coreidae) was high at constant temperatures of 20 to 36°C, and under alternating day (32°C) and night (27°C) temperatures. Hatching percentages ranged from 72.4% at 36°C to 100% at 20°C. These findings are in general agreement with the results obtained in the present study of *N. huttoni*.

Effect of photoperiod on hatching percentage of eggs. Table 3-3 shows the hatching percentages of eggs of *N. huttoni* under three different photoperiods at 20 and 27.5°C. The highest hatching percentage of eggs (91.2%) was obtained under the 12-h photoperiod at both 20 and 27.5°C. Although hatching percentage was lower at both 16-h and 8-h photoperiods, One-way ANOVA did not show significant differences for hatching percentages among the three photoperiods ($P > 0.05$). This indicates that photoperiod had no apparent effect on the hatching percentage of eggs of *N. huttoni*.

Table 3-3. Hatching percentage of eggs of *N. huttoni* under different photoperiods at 20 and 27.5°C.

Temperature (°C)	Photoperiod (L:D)	No. eggs tested	No. eggs hatched	Hatching percentage (% \pm S.E.)
20	16:8	121	96	82.4 \pm 4.9 a
	12:12	148	138	91.2 \pm 3.1 a
	8:16	100	85	86.4 \pm 4.8 a
27.5	16:8	149	131	85.9 \pm 4.6 a
	12:12	156	143	91.2 \pm 2.9 a
	8:16	143	125	89.0 \pm 4.1 a

Hatching percentages of eggs are not significantly different ($P > 0.05$; One-way ANOVA) among different photoperiods at each temperature.

3.3.2 Development of eggs and nymphs

Effect of temperature. The effects of constant and fluctuating temperatures on the development of eggs and nymphs of *N. huttoni* are summarised in Table 3-4. An inverse relationship was found between temperature and duration of development of the egg stage, each instar, nymphal stage and the complete life cycle from egg to adult. Thus, developmental duration of *N. huttoni* at constant temperatures was significantly faster at higher temperatures ($P < 0.01$) (Fig. 3-1). No significant differences ($P > 0.05$) were detected in developmental duration of the egg stage, or 3rd and 4th instars between 25 and 30°C, but for each instar there was a decrease in development time as temperature increased to 30°C. Significantly shorter developmental durations were recorded for eggs, each instar, nymphal stage and total life cycle from egg to adult at 35°C than for those reared at other temperatures ($P < 0.01$). Each successive decrease in temperature resulted in significantly longer developmental duration. The nymphs reared at 35°C completed 5 instars in a mean time of 14.5 days. Developmental duration for the 5 nymphal instars reared at 15, 20, 25, and 30°C were 84.7, 44.5, 22.5,

Table 3-4. Mean developmental duration in days (\pm S.E.) of *N. huttoni* at different temperatures (range in parentheses)

Temperature (°C)	Sex	No. Individuals	Duration						Nymphal duration	Total duration (egg to adult)
			Egg stage	1st instar	2nd instar	3rd instar	4th instar	5th instar		
15	F	8	24.00 \pm 0.53 (22 - 26)	16.38 \pm 1.34 (13 - 23)	13.75 \pm 0.75 (12 - 18)	13.13 \pm 1.01 (11 - 20)	15.88 \pm 0.52 (14 - 18)	24.75 \pm 0.75 (23 - 29)	83.88 \pm 2.03 (77 - 91)	107.88 \pm 2.35 (100 - 116)
	M	9	24.33 \pm 0.58 (21 - 27)	14.00 \pm 0.76 (12 - 19)	14.67 \pm 0.74 (12 - 18)	13.89 \pm 0.79 (11 - 18)	17.44 \pm 0.91 (14 - 23)	25.33 \pm 1.03 (22 - 32)	85.33 \pm 2.83 (73 - 96)	109.67 \pm 2.81 (99 - 120)
	F + M	17	24.18 \pm 0.39 (21 - 27)	15.12 \pm 0.78 (12 - 23)	14.24 \pm 0.51 (12 - 18)	13.53 \pm 0.62 (11 - 20)	16.71 \pm 0.55 (14 - 23)	25.06 \pm 0.63 (22 - 32)	84.65 \pm 1.73 (73 - 96)	108.82 \pm 1.82 (99 - 120)
20	F	32	13.22 \pm 0.15 (11 - 14)	9.63 \pm 0.29 (7 - 14)	6.22 \pm 0.13 (4 - 8)	6.50 \pm 0.16 (5 - 9)	8.75 \pm 0.29 (6 - 13)	13.22 \pm 0.28 (11 - 16)	44.31 \pm 0.68 (38 - 52)	57.53 \pm 0.69 (51 - 66)
	M	43	13.33 \pm 0.14 (11 - 15)	9.42 \pm 0.26 (7 - 14)	6.60 \pm 0.13 (5 - 9)	6.63 \pm 0.17 (4 - 11)	8.70 \pm 0.23 (6 - 13)	13.21 \pm 0.22 (11 - 18)	44.56 \pm 0.56 (38 - 53)	57.88 \pm 0.58 (51 - 67)
	F + M	75	13.28 \pm 0.10 (11 - 15)	9.51 \pm 0.19 (7 - 14)	6.44 \pm 0.09 (4 - 9)	6.57 \pm 0.12 (4 - 11)	8.72 \pm 0.18 (6 - 13)	13.21 \pm 0.18 (11 - 18)	44.45 \pm 0.43 (38 - 53)	57.73 \pm 0.44 (51 - 67)
25	F	18	5.11 \pm 0.16 (4 - 6)	5.06 \pm 0.15 (4 - 6)	2.89 \pm 0.18 (2 - 4)	3.39 \pm 0.14 (3 - 5)	4.61 \pm 0.20 (4 - 7)	7.00 \pm 0.29 (5 - 11)	22.94 \pm 0.45 (20 - 28)	28.06 \pm 0.46 (25 - 33)
	M	14	5.00 \pm 0.18 (4 - 6)	5.00 \pm 0.18 (4 - 6)	2.86 \pm 0.18 (2 - 4)	3.07 \pm 0.07 (3 - 4)	4.43 \pm 0.20 (4 - 6)	6.64 \pm 0.33 (5 - 10)	22.00 \pm 0.44 (20 - 25)	27.00 \pm 0.47 (25 - 30)
	F + M	32	5.06 \pm 0.12 (4 - 6)	5.03 \pm 0.11 (4 - 6)	2.88 \pm 0.13 (2 - 4)	3.25 \pm 0.09 (3 - 5)	4.53 \pm 0.14 (4 - 7)	6.84 \pm 0.22 (5 - 11)	22.53 \pm 0.32 (20 - 28)	27.59 \pm 0.34 (25 - 33)
30	F	39	5.08 \pm 0.04 (5 - 6)	3.36 \pm 0.09 (2 - 5)	2.41 \pm 0.13 (1 - 4)	3.23 \pm 0.21 (1 - 7)	3.97 \pm 0.17 (2 - 7)	5.49 \pm 0.17 (4 - 8)	18.46 \pm 0.29 (15 - 24)	23.54 \pm 0.29 (20 - 29)
	M	33	5.06 \pm 0.04 (5 - 6)	3.39 \pm 0.14 (2 - 6)	2.27 \pm 0.14 (1 - 5)	2.88 \pm 0.19 (2 - 6)	3.76 \pm 0.19 (2 - 7)	5.30 \pm 0.16 (4 - 7)	17.61 \pm 0.37 (14 - 21)	22.67 \pm 0.37 (19 - 27)
	F + M	72	5.07 \pm 0.03 (5 - 6)	3.38 \pm 0.08 (2 - 6)	2.35 \pm 0.09 (1 - 5)	3.07 \pm 0.14 (1 - 7)	3.88 \pm 0.12 (2 - 7)	5.40 \pm 0.12 (4 - 8)	18.07 \pm 0.24 (14 - 24)	23.14 \pm 0.24 (19 - 29)
35	F	91	3.24 \pm 0.05 (3 - 4)	2.88 \pm 0.06 (2 - 4)	2.00 \pm 0.06 (1 - 3)	2.41 \pm 0.06 (1 - 4)	3.11 \pm 0.09 (1 - 6)	4.32 \pm 0.10 (3 - 6)	14.71 \pm 0.20 (11 - 20)	17.96 \pm 0.21 (14 - 24)
	M	103	3.29 \pm 0.05 (3 - 4)	2.94 \pm 0.05 (2 - 5)	2.09 \pm 0.06 (1 - 5)	2.19 \pm 0.06 (1 - 4)	3.07 \pm 0.07 (2 - 5)	4.00 \pm 0.07 (3 - 6)	14.29 \pm 0.17 (11 - 20)	17.58 \pm 0.18 (14 - 23)
	F + M	194	3.27 \pm 0.03 (3 - 4)	2.91 \pm 0.04 (2 - 5)	2.05 \pm 0.04 (1 - 5)	2.29 \pm 0.05 (1 - 4)	3.09 \pm 0.06 (1 - 6)	4.15 \pm 0.06 (3 - 6)	14.49 \pm 0.13 (11 - 20)	17.76 \pm 0.14 (14 - 24)
L-T	F	30	10.47 \pm 0.09 (10 - 11)	6.30 \pm 0.26 (4 - 12)	5.23 \pm 0.24 (3 - 8)	6.83 \pm 0.18 (5 - 9)	8.93 \pm 0.39 (6 - 14)	11.73 \pm 0.32 (10 - 19)	39.03 \pm 0.62 (33 - 47)	49.50 \pm 0.63 (43 - 57)
	M	22	10.68 \pm 0.10 (10 - 11)	6.00 \pm 0.20 (5 - 9)	5.64 \pm 0.30 (3 - 8)	7.00 \pm 0.33 (6 - 12)	8.27 \pm 0.47 (6 - 14)	11.45 \pm 0.33 (9 - 15)	38.36 \pm 0.60 (34 - 46)	49.05 \pm 0.59 (45 - 56)
	F + M	52	10.56 \pm 0.07 (10 - 11)	6.17 \pm 0.17 (4 - 12)	5.40 \pm 0.19 (3 - 8)	6.90 \pm 0.17 (5 - 12)	8.65 \pm 0.30 (6 - 14)	11.62 \pm 0.23 (9 - 19)	38.75 \pm 0.44 (33 - 47)	49.31 \pm 0.44 (43 - 57)

Times required for development of females and males are not significantly different ($P > 0.05$; T-test) at any temperature or for any developmental stage.

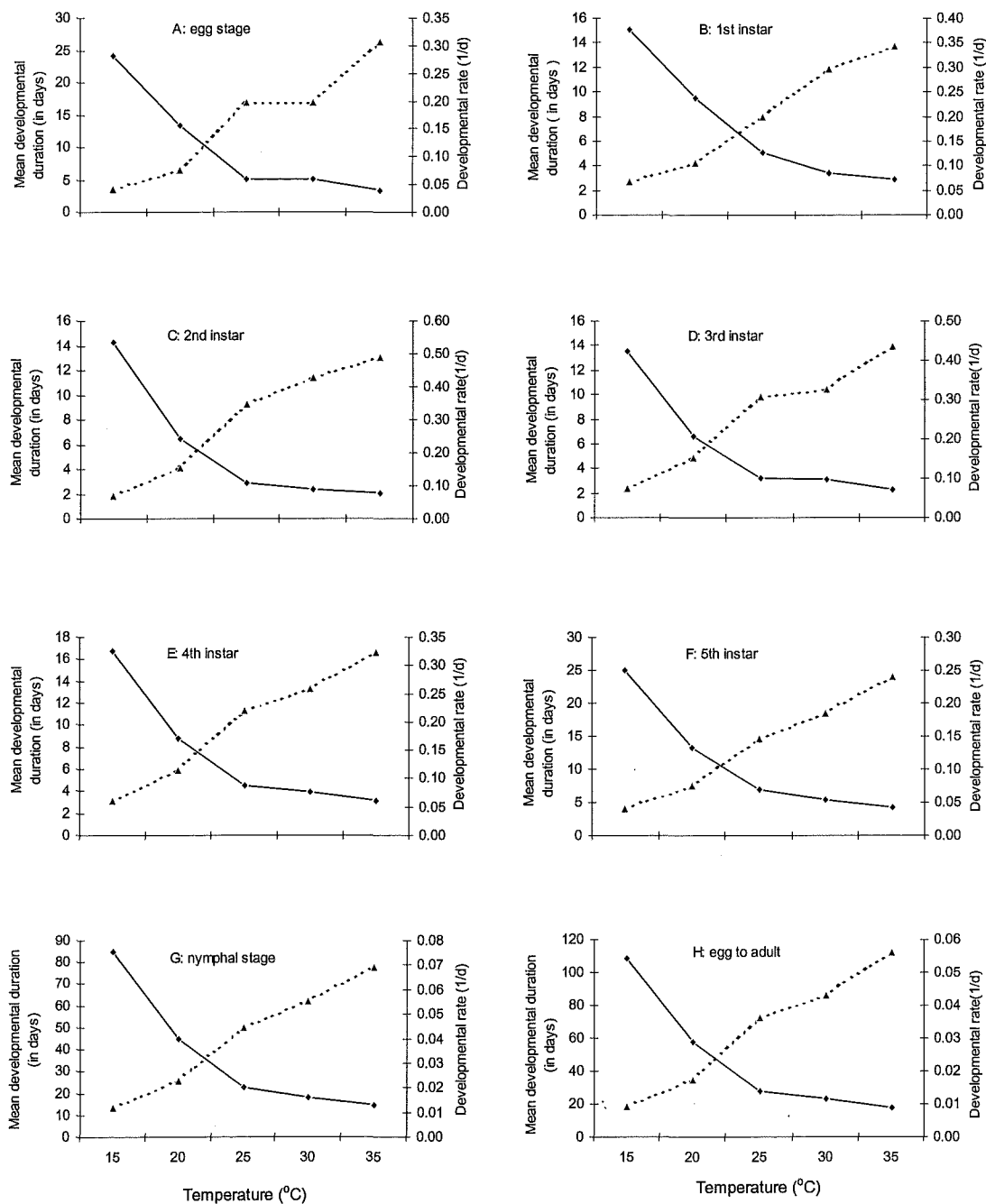


Fig. 3-1. The relationship between temperature and development of *N. huttoni*. Solid line: developmental duration; dashed line: developmental rate.

and 18.1 days, respectively. All developmental stages exhibited a significantly longer developmental duration when reared at 15°C than when reared at other temperatures.

An increase of 5°C at lower temperatures (between 15 and 25°C), however, reduced developmental duration much more than a corresponding increase at higher temperatures (between 25 and 35°C). For instance, total developmental duration (egg to adult) averaged 108.8 days at 15°C, 57.7 days at 20°C and 27.6 days at 25°C, the differences being 51.1 and 30.1 days, respectively for an increase of 5°C. Similarly, the difference in developmental duration was 4.5 days between 25 and 30°C and 5.4 days between 30 and 35°C. Eggs also developed more rapidly at higher temperatures. Thus, the difference in developmental duration was 10.9 days between 15 and 20°C, and 8.2 days between 20 and 25°C. However, there was no difference in developmental duration at 25°C compared with 30°C, and only 1.8 days difference between 30 and 35°C.

Although the average lab temperature of 20.3°C (12.5-29.5°C) was very close to that of the 20°C constant temperature treatment, developmental duration at fluctuating temperature was much shorter than at 20°C. The duration of the egg stage (10.6 days), nymphal duration (38.8 days), and total duration from egg to adult (49.3 days) at fluctuating temperature was shorter by 2.7, 5.7, and 8.4 days, respectively than that at the constant temperature of 20°C (13.3, 44.5, and 57.7 days). This indicates that fluctuating temperature, as is found in the field, is favourable for the development of *N. huttoni*.

No differences ($P > 0.05$, T-tests) in the duration of the egg stage were found between eggs producing male and female nymphs at different constant and fluctuating temperatures. Furthermore, no differences in duration of each instar, nymphal stage, or the total duration (egg to adult) of males and females

were found at any temperature ($P > 0.05$). These results show that the development of females and males of *N. huttoni* is synchronous.

In a complete life cycle, the 5th instar and the egg stage were longest and the 2nd instar was shortest (except at 15°C) at all constant and fluctuating temperatures (Fig. 3-2). Developmental duration for 5th instar nymphs was about twice that of 2nd and 3rd instars, and 1.5 X that of 1st and 4th instars at any given temperature.

The data given in Table 3-4 were used to calculate regression equations and coefficients of determination (r^2) relating temperature to rate of development. Results are given in Table 3-5 and Fig. 3-3. Although Davidson (1942) and Andrewartha & Birch (1954) suggested that a logistic curve should be used for growth data, I found that between 15 and 35°C a linear regression equation provided a good fit, as shown by the high coefficient of determination (r^2) values. These ranged from 0.94 in the egg stage to 0.99 in the 5th instar showed there was a very strong correlation between temperature and developmental rate. My results indicate that every 1°C increase in temperature should result in a corresponding 1.3, 0.29, and 0.24% increase in the rates of development of eggs, nymphal stage, and entire life cycle from egg to adult, respectively. The developmental rate associated with each 1°C increase in temperature was greatest in the 2nd instar (slope $b = 0.0221$) and was least for total development ($b = 0.0024$).

In a similar study of the effect of temperature on the rate of development of the egg and nymphal stages of *Lygus hesperus* Knight (Hemiptera: Miridae), Champlain & Butler (1967) noted that the slope ($b = 0.0034$) of the regression line for nymphal development was about half that ($b = 0.007$) of the egg development line and that, at a given temperature, development of the nymphs required about twice as much time as hatching of eggs. Champlain & Sholdt (1967) reported that in *Geocoris punctipes* (Hemiptera: Lygaeidae), the

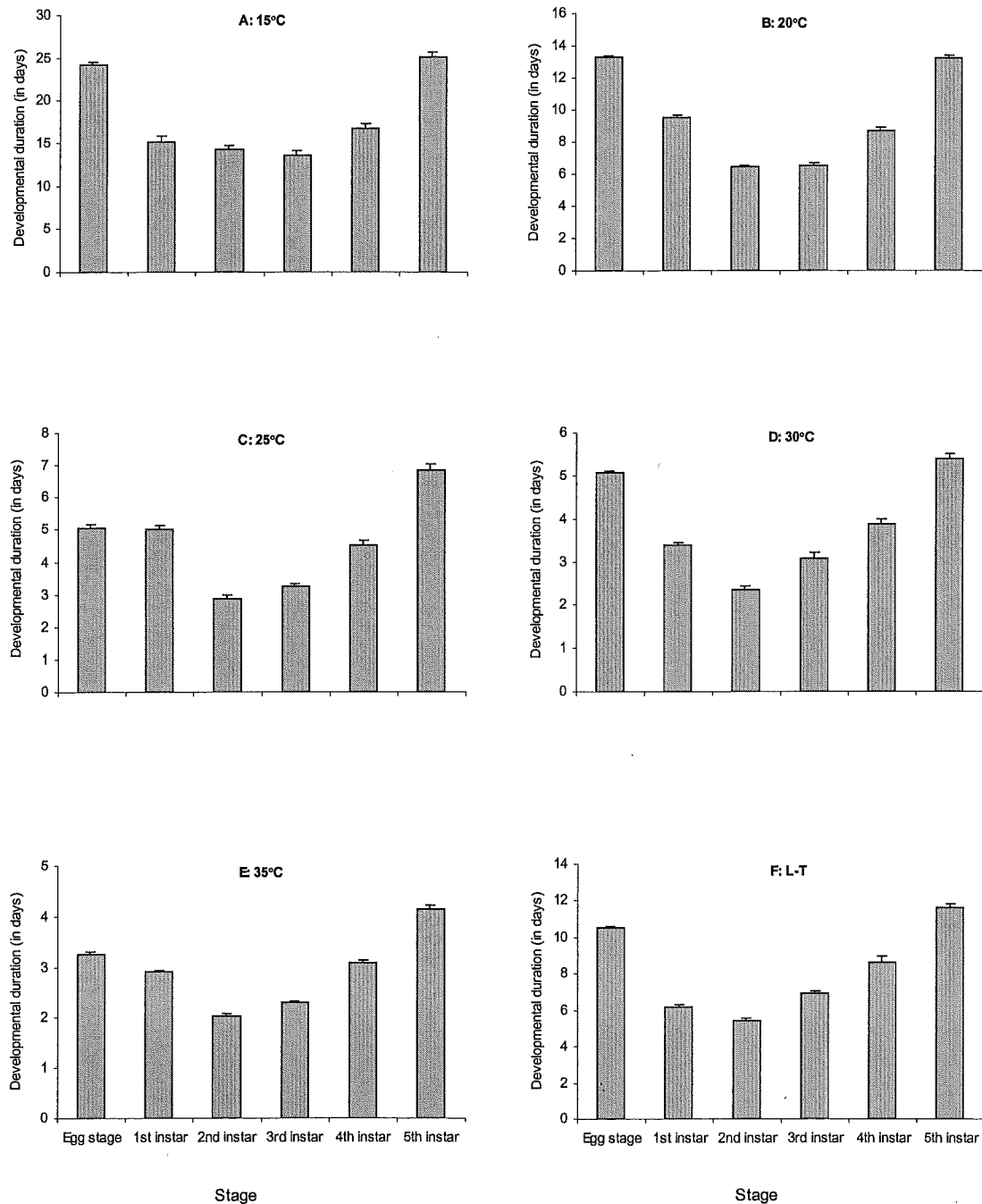


Fig. 3-2 (1). Mean developmental duration in days (+ S.E.) of males and females combined at constant and fluctuating temperatures.

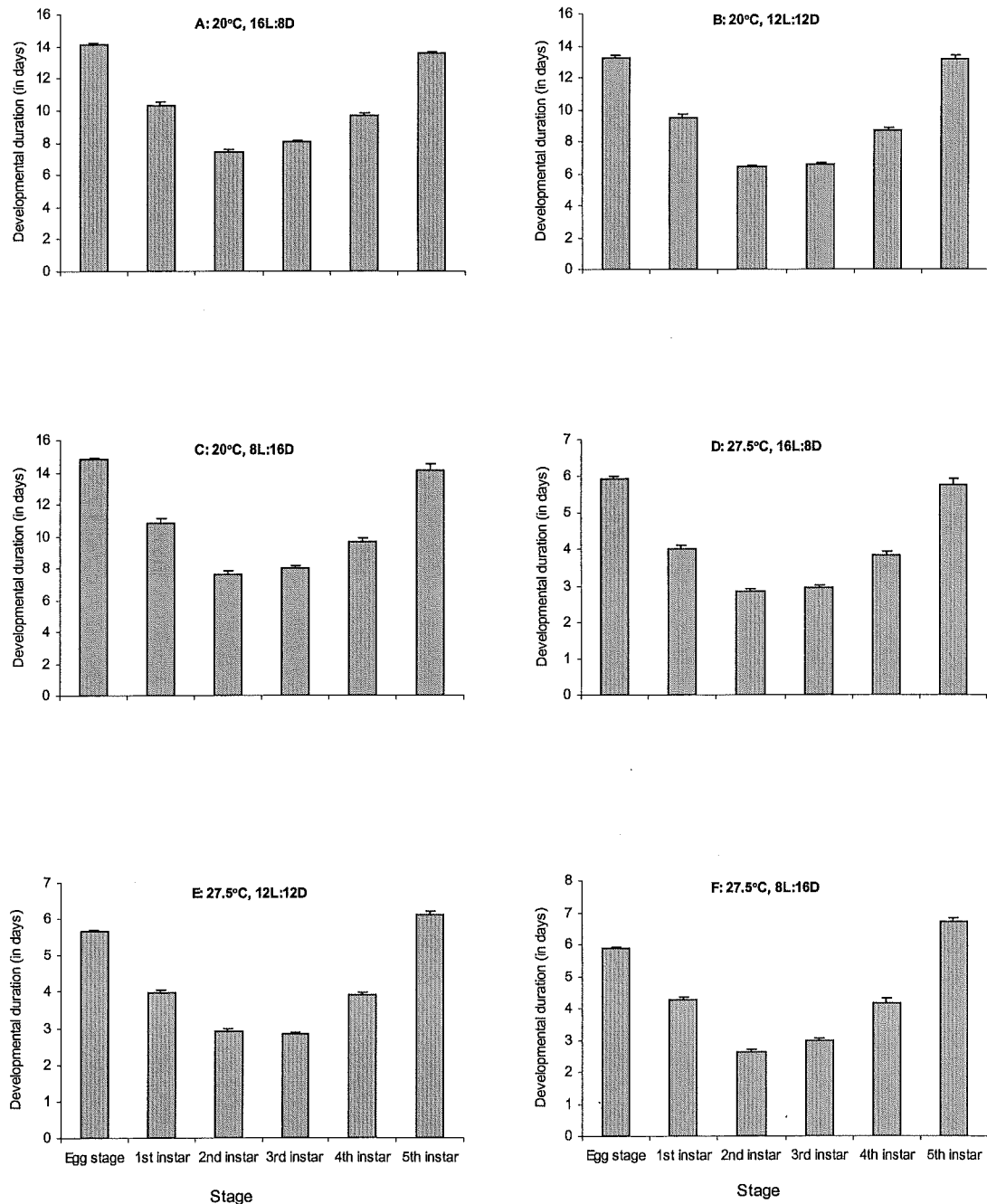


Fig. 3-2 (2). Mean developmental duration in days (± S.E.) of males and females combined under different photoperiods at 20 and 27.5°C.

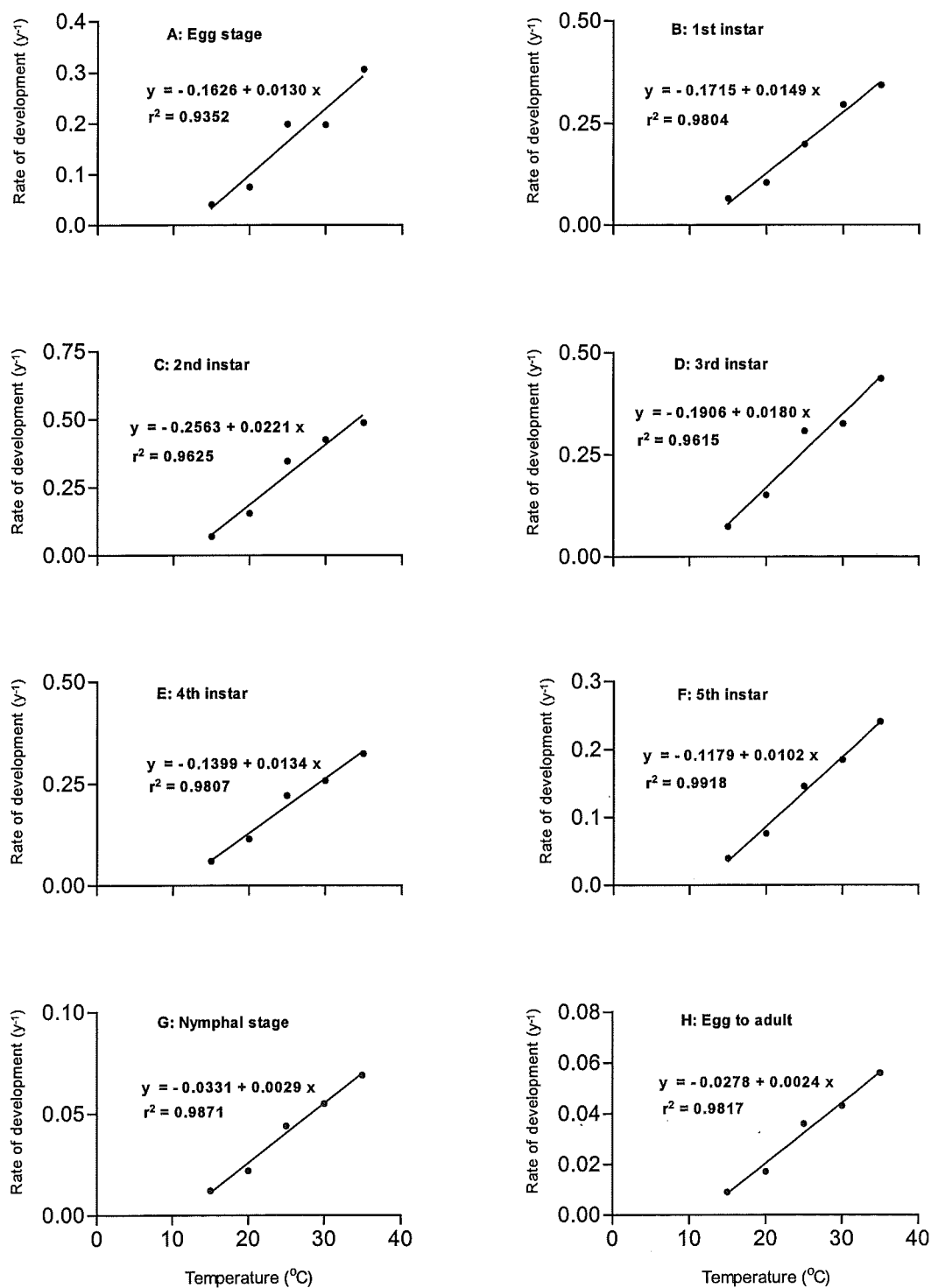


Fig. 3-3. Effect of temperature on rate of development of different life cycle stages of *N. huttoni*.

Table 3-5. Regression equation, r and r^2 values for developmental rate of *N. huttoni* in relation to temperature.

Stage	Regression equation ($y = -a + bX$)	r	r^2	P
Egg stage	$y = -0.1626 + 0.0130 x$	0.9671	0.9352	< 0.01
1st instar	$y = -0.1715 + 0.0149 x$	0.9902	0.9804	< 0.01
2nd instar	$y = -0.2563 + 0.0221 x$	0.9811	0.9625	< 0.01
3rd instar	$y = -0.1906 + 0.0180 x$	0.9806	0.9615	< 0.01
4th instar	$y = -0.1399 + 0.0134 x$	0.9903	0.9807	< 0.01
5th instar	$y = -0.1179 + 0.0102 x$	0.9959	0.9918	< 0.01
Nymphal stage	$y = -0.0331 + 0.0029 x$	0.9935	0.9871	< 0.01
Egg to adult	$y = -0.0278 + 0.0024 x$	0.9908	0.9817	< 0.01

Regression equation $y = a + bX$, where y is reciprocal of mean developmental duration and X is temperature. r is coefficient of correlation and r^2 is coefficient of determination. When degrees of freedom $V = 3$, $r_{0.05} = 0.878$; and $r_{0.01} = 0.959$. All r values $> r_{0.01} = 0.959$, so $P < 0.01$.

slope ($b = 0.00248$) of the regression line for nymphal development was 1/3 that ($b = 0.00751$) of the egg development line, an indication that only about 1/3 as much nymphal development occurred per unit temperature as in egg development. In the present study, the rates of development of the egg stage of *N. huttoni* ($b = 0.0130$) and the nymphal stage ($b = 0.0029$) were compared (Table 3-5). The slope of the regression line describing nymphal development was about 1/4 that of the egg development line, and development of nymphs, at any given temperature, required about 4 times as much time as egg development. The mean ratio of egg developmental time/nymphal developmental time, or the slope of the nymphal stage/slope of egg stage were found to be about 3/4 in *Hypera brunneipennis* (Madubunyi & Koehler 1974), *Geocoris atricolor*, *G. pallens*, and *G. punctipes* (Dunbar & Bacon 1972), and 1/5 in *Spodoptera litura* (Rao *et al.* 1989).

These studies confirm that temperature is one of the most important factors influencing the development of insects. In *N. huttoni*, a direct relationship exists between temperature and rate of development. A difference of 5°C at

lower temperatures (15-25°C) affected developmental duration much more than a corresponding difference at higher temperatures (25-35°C). Progressive increases of 5°C in temperature from 15-25°C resulted in about 50% reduction in developmental time, while corresponding increases in temperature from 25-30°C resulted in about 20% reduction in developmental time. These findings indicate that a deceleration in the speed of development started above 25°C. Development of eggs, nymphs, and egg to adult of *N. vinitor* (Kehat & Wyndham 1972a) displayed very similar variations in reduction in developmental time as temperature increased progressively by 5°C from 20–35°C. In their study, developmental duration was reduced about 50% between 20 and 25°C, 34% between 25 and 30°C, and 20% between 30 and 35°C. Madubunyi & Koehler (1974) studied the development of *Hypera brunneipennis* at five constant temperatures (15, 20, 25, 30, and 35°C). They found that progressive 5°C temperature increments from 15–25°C resulted in corresponding halving of developmental time, and corresponding temperature increments from 25–35°C brought about a 20% reduction in development time. The findings on *N. vinitor* and *H. brunneipennis* are in agreement with the results presented in my study.

Differences in rates of development among individuals within the same stage at any given temperature were less at 25-35°C than at 15-20°C (Table 3-4), with the greatest differences occurring at 15°C. Differences in developmental rate among individuals were probably due to inherent properties of the individuals and perhaps differences in feeding.

The mean incubation time of eggs at any given temperature was longer than the mean developmental duration of any one of the 1st–4th instars, but was very similar to or slightly shorter than that of the 5th instar (Table 3-4). Variation in developmental duration of instars at any given temperature followed an identical and regular order very clearly: declining from the first to

the second instar – the shortest one- and then increasing gradually from the second to the 5th instar (Fig 3-2 (1)). An exception was found at 15°C where the 3rd instar was the shortest. Similarly, developmental times of the 2nd instars of *Nabis americanoferus* & *N. roseipennis* (Braman et al. 1984), *Gerris paludum insularis* (Park 1988), and *Corythucha morrilli* (Stone & Watterson 1985) were reported to be the shortest. The data available show that in Hemiptera, the instar with the shortest developmental time differs among species and at different temperatures. For example, the shortest instar was the 1st in the green stink bug, *Acrosternum hilare* (Simmons & Yeargan 1988), *Biprorulus bibax* (James 1990), and *Acanthomia tomentosicollis* Stal (Egwuatu & Taylor 1977); the 3rd in *Corythucha cydoniae* (Neal & Douglass 1990), *Stephanitis pyrioides* (Neal & Douglass 1988, Braman et al. 1992), and *Orius insidiosus* (Isenhour & Yeargan 1981); and the 2nd and 3rd in *Orius tristicolor* (Askari & Stern 1972).

Mean developmental duration of the nymphal stages in the present study at 20°C (female: 44.3 days; male: 44.6 days) and 25°C (female: 22.9 days; male: 22.0 days) were similar to results obtained by Kehat & Wyndham (1972a) at 20°C (female: 45.1 days; male: 45.5 days) and 25°C (female: 22.5 days; male: 22.1 days) for *Nysius vinitor*. However, the mean developmental duration for *N. huttoni* at 30°C (female: 18.5 days; male: 17.6 days) and 35°C (female: 14.7 days; male: 14.3 days) was longer than that of *N. vinitor* at 30°C (female: 14.5 days; male: 14.4 days) and 35°C (female: 11.4 days; male: 11.3 days). This indicates that *N. vinitor* developed faster than *N. huttoni* at temperatures above 30°C.

The mean incubation time of eggs of *N. huttoni* at 20°C was 13.3 days, at 25°C 5.1 days, at 30°C 5.1 days and at 35°C 3.3 days (Table 3-4), while in *N. vinitor* at 20°C it was 12.5 days, at 25°C 6.5 days, at 30°C 4.5 days and at 35°C 3.7 days (Kehat & Wyndham 1972a). Thus, there was a slight difference

(0.8, -1.4, 0.6, and -0.4 days, respectively) between the two species at these four constant temperatures. At 15°C, however, the mean incubation time of 36.8 days recorded for *N. vinitor* by Kehat & Wyndham (1972a) was longer by 12.6 days than that of 24.2 days recorded for *N. huttoni* in the present study. The reason for this may be that the eggs tested in the present study were laid at 30°C and then transferred to 15°C to develop. Kehat & Wyndham (1972a) found that total developmental duration (from egg to adult) of *N. vinitor* required 57.6, 29.0, 19.0 and 15.1 days at constant temperatures of 20, 25, 30 and 35°C, respectively. These times are comparable to the values for total duration of development from egg to adult that I obtained for *N. huttoni* (Table 3-4).

Every species has certain temperature limits above and below which development is retarded, or at extremes, at which death ensues. Some insects are able to escape lethal temperatures by migrating, but the habitat ranges of most insect species are limited by the temperature regime to which they are individually adapted. Different insect species have different upper and lower temperature limits for development, and even different populations or different developmental stage of one species vary in their ability to withstand lethal lower and higher temperatures.

As mentioned above, no rearing was carried out below 15°C or above 35°C, so no information on development at extremely low and high temperatures was obtained in my study. However, as indicated earlier, Eyles (1963b) kept 4th and 5th instar nymphs of *N. huttoni* at high temperatures between 34-47°C. Ten of 15 at 34°C, 7 of 10 at 34.5°C and 5 of 10 at 36.3°C completed growth and moulting, and emerged as adults, but growth and moulting to adults did not occur above 41.7°C. This indicates that the lethal temperature for nymphs of *N. huttoni* may be in the range 38-40°C. At a low temperature of 6°C, Eyles kept sixteen 4th instar nymphs. Six nymphs moulted to the 5th

instar, but none to adult. Of those that did not moult, five survived less than 36 days, three survived 36 days, and two survived 40 days. Of those that moulted, two survived 36 days, two 42 days and two 48 days. Although not one became adult, survival was prolonged. These data indicate that *N. huttoni* is able to tolerate extended periods of temperature as low as 6°C. However, in my study, the threshold temperatures for development of 4th and 5th instar nymphs were respectively $10.7 \pm 1.0^{\circ}\text{C}$ and $11.1 \pm 0.7^{\circ}\text{C}$ (Table 3-9). From these two studies, I concluded that the effective lower limit for development of nymphs of *N. huttoni* is about 11°C, although they are able to tolerate lower temperatures.

Robert & Butler (1967) studied the development of eggs and nymphs of *Lygus hesperus* (Hemiptera: Miridae) at 7 constant temperatures of 10, 15, 20, 25, 30, 35 and 40°C. They found that a constant temperature of 40°C was lethal to both eggs and nymphs, whereas a temperature of 10°C was lethal to eggs but not nymphs. Thus, 6 nymphs completed development at 10°C, although the time required was almost 3 months and 6 individuals died the day they became adults. In *N. huttoni*, the lower temperature limit for development of eggs and nymphs was about 11°C, while the upper temperature limit of 45°C for development of eggs was a little higher than that of about 42°C for nymphal development (Eyles 1963b). These data demonstrate that the lower and upper temperature limits for egg development are not necessarily congruent with the lower or upper temperature limits for nymphal development. Considerable variation may also be found in response to temperature in other species of Hemiptera. Stone & Waterson (1985), for example, considered 17.8°C and 34.4°C to be the effective lower and upper limits, respectively for development of *Corythucha morrilli* Osborn and Drake. Park (1988) estimated that the lower developmental temperature threshold of *Gerris paludum insularis* was 11°C and the upper, 35.6°C. James (1990) found that eggs of *Biprorulus bibax* hatched after 16.2 and 2.4 days at 17.5 and 35°C, respectively, but nymphs

failed to develop beyond the second instar at these temperatures. Successful development through all instars occurred at 20-32.5°C, however.

Results obtained at lab temperature fluctuating between 12.5°C and 29.5°C in my study are illuminating. Since the mean developmental duration of 49.3 days (egg to adult) recorded in the lab at an average temperature of 20.3°C was 8.4 days shorter than that recorded at a constant temperature of 20°C (57.7 days). This suggests that the increase in the rate of development at higher temperatures (20–29.5°C) was greater than the decrease in the rate of development resulting from lower temperatures (12.5-20°C). Because the minimum lab temperature of 12.5°C was higher than the lower threshold temperature for development of $11.1 \pm 0.8^\circ\text{C}$ (Table 3-9), development did not stop actually, but would have only slowed down between 12.5 and 20°C.

Developmental rates in the field might be expected to be slightly faster or slower than those observed under constant temperatures if field temperatures fall below the lower developmental threshold or rise above the upper developmental threshold (Higley *et al.* 1986). Field temperatures in Canterbury frequently fall below the lower developmental threshold of 11.4°C for development from egg to adult during spring, and ground temperatures often exceed 38-40°C during summer. Therefore, it is possible that field populations of *N. huttoni* might develop faster or slower than the laboratory population kept at ambient temperature. Nevertheless, developmental data presented here provide a basis for predicting the phenology of egg and nymphal populations in the field for *N. huttoni*.

Thus, *N. huttoni* can develop successfully and occur where there are a minimum of 419.1 day-degrees above 11.4°C, provided suitable host plants are available. In much of Canterbury, this basic heat requirement can be

satisfied, and seems to explain in part the occurrence of *N. huttoni* over a wide geographical range.

In the present study, no differences were found in developmental duration between females and males of any instar, nymphal stage or over the complete life cycle of *N. huttoni* at any given temperature. This is consistent with the findings reported by Kehat & Wyndham (1972a) for *N. vinitor*. Similar results were reported for *Corythucha morrilli* Osborn (Stone & Watterson 1985), *Stephanitis pyrioides* (Near & Douglass 1988, Braman *et al.* 1992), *Corythucha cydoniae* (Neal & Douglass 1990), the green stink bug, *Acrosternum hilare* (Simmons & Yeargan 1988), and *Biprorulus bibax* (James 1990).

Effect of photoperiod. The effect of photoperiod on development of *N. huttoni* was examined at 20 and 27.5°C and the results are presented in Table 3-6. Time required for development of females and males did not differ significantly ($P > 0.05$, T-test) under three photoperiods at either temperature. Time required for the development of the 2nd instar was least, while the 5th instar and egg stage required the longest time as shown in Fig. 3-2 (2). These results are in accordance with those obtained at different temperatures.

Development of *N. huttoni* was significantly related to photoperiod. At 20°C, eggs, each instar, the nymphal stage and the complete life cycle developed significantly faster when kept under a 12-h photoperiod than at 16-h and 8-h photoperiods ($P < 0.05$ or 0.01, One-way ANOVA). Only the egg stage and the total duration from egg to adult were significantly longer at the 16-h photoperiod than at the 8-h photoperiod ($P < 0.05$ or 0.01). Nymphal development was not significantly different under the two photoperiods ($P > 0.05$). These results indicated that both long-day (≥ 16 hours) and short-day photoperiods (≤ 8 hours) prolonged developmental time of *N. huttoni* at 20°C, and that the latter prolonged it more than the former.

Table 3-6. Mean developmental duration in days (\pm S.E.) of *N. huttoni* under different photoperiods at 20 and 27.5°C (range in parentheses).

Temperature (°C)	Photoperiod (L:D)	Sex	No. individuals	Duration						Nymphal duration	Total duration (egg to adult)
				Egg stage	1st instar	2nd instar	3rd instar	4th instar	5th instar		
20	16:8	F	42	14.12 \pm 0.09 (13-15)	10.33 \pm 0.22 (7-13)	7.29 \pm 0.15 (5-9)	8.00 \pm 0.15 (6-10)	9.81 \pm 0.21 (8-14)	13.69 \pm 0.18 (12-17)	49.12 \pm 0.35 (45-54)	63.24 \pm 0.38 (59-69)
		M	29	14.14 \pm 0.13 (13-16)	10.24 \pm 0.40 (7-16)	7.66 \pm 0.22 (6-10)	8.03 \pm 0.18 (7-10)	9.52 \pm 0.22 (8-12)	13.34 \pm 0.25 (11-17)	48.79 \pm 0.62 (42-54)	62.93 \pm 0.62 (56-68)
		F+M	71	14.13 \pm 0.07 (13-16)	10.30 \pm 0.21 (7-16)	7.44 \pm 0.13 (5-10)	8.01 \pm 0.11 (6-10)	9.69 \pm 0.15 (8-14)	13.55 \pm 0.14 (11-17)	48.99 \pm 0.33 (42-54)	63.11 \pm 0.34 (56-69)
	12:12	F	32	13.22 \pm 0.15 (11-14)	9.63 \pm 0.29 (7-14)	6.22 \pm 0.13 (4-8)	6.50 \pm 0.16 (5-9)	8.75 \pm 0.29 (6-13)	13.22 \pm 0.28 (11-16)	44.31 \pm 0.68 (38-52)	57.53 \pm 0.69 (51-66)
		M	43	13.33 \pm 0.14 (11-15)	9.42 \pm 0.26 (7-14)	6.60 \pm 0.13 (5-9)	6.63 \pm 0.17 (4-11)	8.70 \pm 0.23 (6-13)	13.21 \pm 0.22 (11-18)	44.56 \pm 0.56 (38-53)	57.88 \pm 0.58 (51-67)
		F+M	75	13.28 \pm 0.10 (11-15)	9.51 \pm 0.19 (7-14)	6.44 \pm 0.09 (4-9)	6.57 \pm 0.12 (4-11)	8.72 \pm 0.18 (6-13)	13.21 \pm 0.18 (11-18)	44.45 \pm 0.43 (38-53)	57.73 \pm 0.44 (51-67)
	8:16	F	12	14.83 \pm 0.21 (14-16)	10.75 \pm 0.53 (9-14)	8.17 \pm 0.51 (7-12)	8.08 \pm 0.38 (6-10)	9.92 \pm 0.40 (8-12)	13.67 \pm 0.54 (11-17)	50.58 \pm 1.22 (47-60)	65.42 \pm 1.20 (61-75)
		M	24	14.79 \pm 0.15 (14-17)	10.92 \pm 0.32 (9-14)	7.29 \pm 0.33 (4-11)	7.92 \pm 0.25 (6-11)	9.46 \pm 0.29 (7-13)	14.38 \pm 0.42 (11-19)	49.96 \pm 0.95 (43-58)	64.75 \pm 1.00 (58-74)
		F+M	36	14.81 \pm 0.12 (14-17)	10.86 \pm 0.27 (9-14)	7.58 \pm 0.28 (4-12)	7.97 \pm 0.21 (6-11)	9.61 \pm 0.24 (7-13)	14.14 \pm 0.33 (11-19)	50.17 \pm 0.74 (43-60)	64.97 \pm 0.77 (58-75)
	16:8 vs 12:12	F+M	P	< 0.001	< 0.05	< 0.001	< 0.001	< 0.001	> 0.05	< 0.001	< 0.001
	16:8 vs 8:16	F+M	P	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05
	12:12 vs 8:16	F+M	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.01	< 0.001	< 0.001
27.5	16:8	F	48	5.96 \pm 0.06 (5-7)	3.98 \pm 0.10 (3-6)	3.00 \pm 0.09 (2-5)	2.92 \pm 0.09 (2-4)	4.04 \pm 0.15 (2-6)	5.79 \pm 0.24 (4-10)	19.73 \pm 0.32 (16-25)	25.69 \pm 0.33 (22-31)
		M	48	5.94 \pm 0.06 (5-7)	4.08 \pm 0.13 (3-7)	2.69 \pm 0.09 (2-5)	3.00 \pm 0.10 (2-5)	3.63 \pm 0.14 (2-6)	5.77 \pm 0.15 (4-9)	19.17 \pm 0.28 (16-24)	25.10 \pm 0.29 (22-30)
		F+M	96	5.95 \pm 0.04 (5-7)	4.03 \pm 0.08 (3-7)	2.84 \pm 0.06 (2-5)	2.96 \pm 0.07 (2-5)	3.83 \pm 0.10 (2-6)	5.78 \pm 0.14 (4-10)	19.45 \pm 0.21 (16-25)	25.40 \pm 0.22 (22-31)
	12:12	F	40	5.60 \pm 0.08 (5-6)	4.00 \pm 0.12 (3-6)	2.93 \pm 0.13 (1-6)	2.88 \pm 0.09 (2-5)	3.83 \pm 0.11 (3-5)	6.25 \pm 0.20 (4-11)	19.88 \pm 0.28 (17-25)	25.48 \pm 0.30 (22-31)
		M	66	5.70 \pm 0.06 (5-7)	3.97 \pm 0.11 (3-7)	2.91 \pm 0.06 (2-4)	2.82 \pm 0.07 (2-4)	3.95 \pm 0.10 (3-6)	6.02 \pm 0.15 (4-11)	19.67 \pm 0.23 (16-26)	25.36 \pm 0.23 (21-32)
		F+M	106	5.66 \pm 0.05 (5-7)	3.98 \pm 0.08 (3-7)	2.92 \pm 0.06 (1-6)	2.84 \pm 0.05 (2-5)	3.91 \pm 0.07 (3-6)	6.10 \pm 0.12 (4-11)	19.75 \pm 0.18 (16-26)	25.41 \pm 0.18 (21-32)
	8:16	F	39	5.97 \pm 0.09 (5-7)	4.26 \pm 0.10 (3-6)	2.67 \pm 0.11 (2-4)	3.05 \pm 0.12 (2-5)	4.23 \pm 0.18 (3-7)	6.95 \pm 0.20 (5-10)	21.15 \pm 0.32 (18-25)	27.13 \pm 0.37 (23-32)
		M	39	5.77 \pm 0.09 (5-7)	4.26 \pm 0.13 (3-7)	2.62 \pm 0.12 (1-5)	2.92 \pm 0.13 (2-6)	4.10 \pm 0.20 (2-7)	6.44 \pm 0.20 (5-11)	20.33 \pm 0.35 (17-26)	26.10 \pm 0.38 (23-32)
		F+M	78	5.87 \pm 0.06 (5-7)	4.26 \pm 0.08 (3-7)	2.64 \pm 0.08 (1-5)	2.99 \pm 0.09 (2-6)	4.17 \pm 0.13 (2-7)	6.69 \pm 0.14 (5-11)	20.74 \pm 0.24 (17-26)	26.62 \pm 0.27 (23-32)
	16:8 vs 12:12	F+M	P	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
	16:8 vs 8:16	F+M	P	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.001	< 0.001	< 0.001
	12:12 vs 8:16	F+M	P	< 0.05	> 0.05	< 0.05	> 0.05	> 0.05	< 0.01	< 0.01	< 0.001

P > 0.05 (T-test) between males and females under any photoperiod and temperature or for any developmental stage. One-way ANOVA was used to test the difference of the mean developmental duration among photoperiods.

At 27.5°C, the photoperiodic effect on development was not so apparent as at 20°C. There was no significant difference ($P > 0.05$) in development duration between 16-h and 12-h photoperiods except on the eggs which developed faster under the latter photoperiod. Developmental duration under the 16-h and 8-h photoperiods was not significantly different ($P > 0.05$) for the egg stage and earlier instars (1st- 4th instar), but the development of the 5th instar, total nymphal stage, and the entire life cycle from egg to adult was significantly slower ($P < 0.01$) under the 8-h than 16-h photoperiod. A similar effect was found between 12-h and 8-h photoperiods.

My results showed that a 16-h long-day photoperiod had an apparent effect on development of *N. huttoni* at the lower temperature (20°C), but not at the higher temperature (27.5°C), and that the effect of an 8-h short-day photoperiod on development was greater at lower temperature (20°C) than at higher temperature (27.5°C). It can be concluded that temperature had a greater effect on development of *N. huttoni* than photoperiod under high temperature condition (27.5°C), and that the effect of an 8-h short-day photoperiod on development of *N. huttoni* was greater than a 16-h long-day photoperiod.

Askari & Stern (1972) studied the effect of photoperiod on developmental duration in *Orius tristicolor* at 25.5°C. They found that the number of days required for *O. tristicolor* to complete nymphal development under 16-h, 12-h and 4-h photoperiods was 14.1, 12.3 and 14.5 days, respectively. In their study photoperiod had a very similar effect on development of nymphs as it did on *N. huttoni*. My findings at 20°C indicated that the 12-h photoperiod resulted in slightly shortened nymphal developmental duration (44.5 days) in comparison to the other photoperiods (49 and 50.2 days at 16-h and 8-h photoperiods, respectively). However, Ruberson *et al.* (1991) reported that development of nymphs of *Orius insidiosus* at 20°C was significantly faster

under a 10-h photoperiod than under 12-h, 13-h, 14-h and 15-h photoperiods. Development of *O. insidiosus* was accelerated at short day-lengths. Clearly, photoperiod has different effects on different species of insects.

In the field, the development of insects is not only affected by temperature and photoperiod but by a combination of these two factors as well as by other factors including humidity, food availability and quality.

3.3.3 Mortality of nymphs

Temperature and mortality of nymphs. Mortality of nymphs of *N. huttoni* at constant and fluctuating temperatures in the laboratory is shown in Table 3-7. Mortality was higher in early instars than later instars at each temperature. The highest nymphal mortality occurred in the first instar and mortality decreased in subsequent instars (Fig. 3-4). Thus, at 25, 30, 35°C and lab temperature, over 50% of nymphs died in the first instar and at 15 and 20°C, over 60% died in the first instar. This shows that even though field populations of newly hatched nymphs may be very high initially, deaths of nymphs is likely to occur mainly in the 1st instar.

Low temperature (15°C) and high temperature (round 35°C) had detrimental and favourable effects, respectively, on the survivorship of nymphs. Total generation mortality at 15°C was 87.3%, at 35°C it was 13.4%, whereas at 20, 25 and 30°C, mortality was 43.2, 56.1 and 44.9%. Total mortality of nymphs at fluctuating temperature was 50%. Because developmental duration from egg to adult was shortest at 35°C (17.8 days) and mortality of nymphs was lowest (13.4%) at this temperature it is apparent that *N. huttoni* is well adapted to life at high temperature.

Table 3-7. Mortality of nymphs of *N. huttoni* at different temperatures.

Temperature (°C)		1st instar	2nd instar	3rd instar	4th instar	5th instar	Adult	Total
15	Population	134	63	52	37	30	17	----
	No. dead nymphs	71	11	15	7	13	----	117
	Generation mortality (%)	53.0	8.2	11.2	5.2	9.7	----	87.3
20	Population	132	93	81	78	75	75	----
	No. dead nymphs	39	12	3	3	0	----	57
	Generation mortality (%)	29.5	9.1	2.3	2.3	0.0	----	43.2
25	Population	107	72	63	56	51	47	----
	No. dead nymphs	35	9	7	5	4	----	60
	Generation mortality (%)	32.7	8.4	6.5	4.7	3.7	----	56.1
30	Population	185	142	127	112	104	102	----
	No. dead nymphs	43	15	15	8	2	----	83
	Generation mortality (%)	23.2	8.1	8.1	4.3	1.1	----	44.9
35	Population	224	208	204	202	198	194	----
	No. dead nymphs	16	4	2	4	4	----	30
	Generation mortality (%)	7.1	1.8	0.9	1.8	1.8	----	13.4
L-T	Population	240	175	150	134	127	120	----
	No. dead nymphs	65	25	16	7	7	----	120
	Generation mortality (%)	27.1	10.4	6.7	2.9	2.9	----	50.0

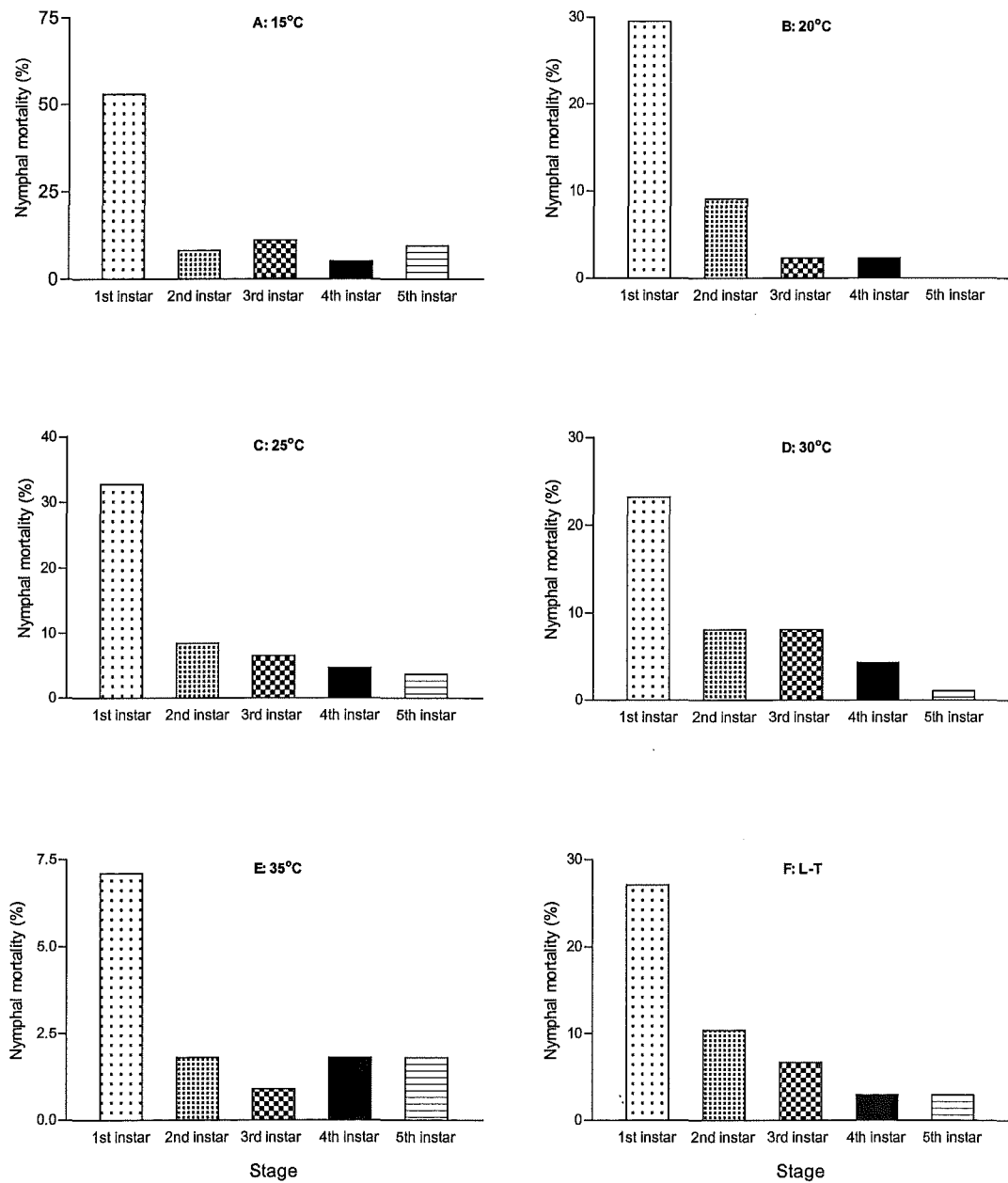


Fig. 3-4. Average percentage mortality of nymphs at constant and fluctuating temperatures.

Photoperiod and mortality of nymphs. Mortality of nymphs of *N. huttoni* under 16-h, 12-h and 8-h photoperiods at 20 and 27.5°C is given in Table 3-8 and Figs 3-5 and 3-6. Greatest mortality was incurred by 1st instar nymphs and mortality decreased in subsequent instars (Fig. 3-5). At 20°C, nymphs that died in the first instar under 16-h, 12-h, and 8-h photoperiods accounted for 72.0, 68.4, and 77.8% respectively, of the nymphs that died in the entire nymphal stage. At 27.5°C, the corresponding percentages were 66.7, 87.1, and 69.7%, respectively. In the first instar, the highest mortality occurred under an 8-h photoperiod at both 20°C (43.2%) and 27.5°C (20.7%) (Fig. 3-5); the lowest mortalities under the 16-h photoperiod were 18.8% at 20°C and 14.6% at 27.5°C. The highest total mortalities of nymphs (1st to 5th instar) were 55.6% at 20°C and 29.7% at 27.5°C and occurred under the 8-h photoperiod (Table 3-8). In contrast, the lowest total mortalities, 26.0% at 20°C and 22.0% at 27.5°C, occurred under the 16-h photoperiod (Fig. 3-6). These results indicate that long-day photoperiods tend to favour survivorship of nymphs of *N. huttoni*.

The results given in Table 3-8 show that mortality of nymphs under three photoperiods was higher at 20°C than at 27.5°C. This further confirmed and supported earlier results on mortality obtained at different temperatures and a photoperiod of 12L:12D. From these experiments, I conclude that overall mortality of nymphs of *N. huttoni* is lowest under high temperature and long-day photoperiodic conditions, whereas short-days especially in combination with low temperature can cause high nymphal mortality.

3.3.4 Threshold temperature and thermal constant of *N. huttoni*

The data in Table 3-4 were used to calculate threshold temperatures and thermal constants, which are given in Table 3-9. The threshold temperatures (C) for development of eggs, nymphs, and the complete life cycle (egg to

Table 3-8. Mortality of nymphs of *N. huttoni* under different photoperiods at 20 and 27.5°C.

Temperature (°C)	Photoperiod (L:D)		1st instar	2nd instar	3rd instar	4th instar	5th instar	Adult	Total
20	16:8	Population	96	78	75	73	72	71	—
		No. dead nymphs	18	3	2	1	1	—	25
		Generation mortality (%)	18.8	3.1	2.1	1.0	1.0	—	26.0
	12:12	Population	132	93	81	78	75	75	—
		No. dead nymphs	39	12	3	3	0	—	57
		Generation mortality (%)	29.5	9.1	2.3	2.3	0.0	—	43.2
	8:16	Population	81	46	40	38	37	36	—
		No. dead nymphs	35	6	2	1	1	—	45
		Generation mortality (%)	43.2	7.4	2.5	1.2	1.2	—	55.6
27.5	16:8	Population	123	105	103	101	99	96	—
		No. dead nymphs	18	2	2	2	3	—	27
		Generation mortality (%)	14.6	1.6	1.6	1.6	2.4	—	22.0
	12:12	Population	137	110	109	108	108	106	—
		No. dead nymphs	27	1	1	0	2	—	31
		Generation mortality (%)	19.7	0.7	0.7	0.0	1.5	—	22.6
	8:16	Population	111	88	83	80	79	78	—
		No. dead nymphs	23	5	3	1	1	—	33
		Generation mortality (%)	20.7	4.5	2.7	0.9	0.9	—	29.7

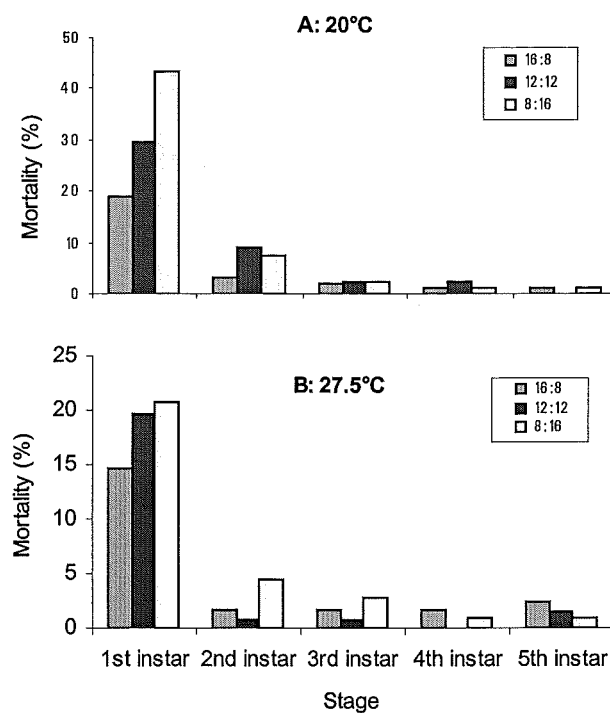


Fig. 3-5. Mortality of nymphs in each instar under different photoperiods at 20 and 27.5°C.

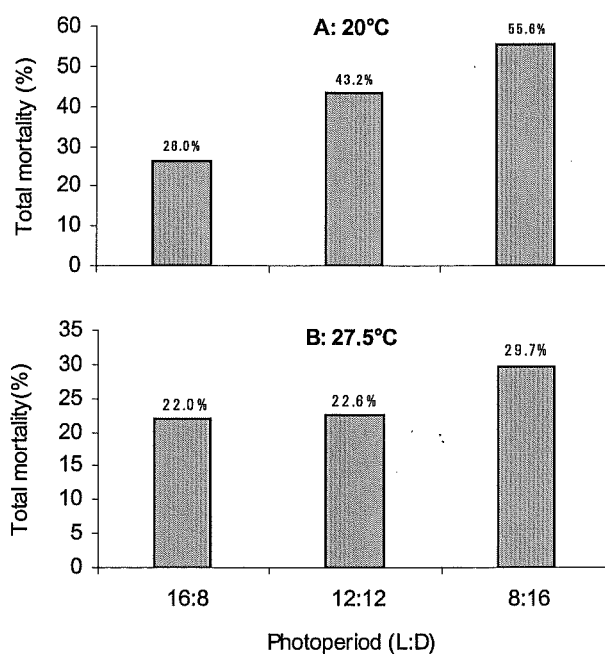


Fig. 3-6. Total mortality of nymphs (1st to 5th instar) under different photoperiods at 20 and 27.5°C.

Table 3-9. Threshold temperature (C) and thermal constant (K) of *N. huttoni*.

	Egg stage	1st instar	2nd instar	3rd instar	4th instar	5th instar	Nymphal stage	Egg to adult
K (DD)	71.8	65.7	43.5	53.5	73.1	96.7	341.3	419.1
C ± S.E. (°C)	13.3 ± 1.8	11.7 ± 1.0	12.1 ± 1.4	11.1 ± 1.4	10.7 ± 1.0	11.7 ± 0.7	11.1 ± 0.8	11.4 ± 1.0

adult) were 13.3, 11.1, and 11.4°C, respectively. The threshold temperatures differed only slightly among different developmental stages. For example, there was a difference of only 2.6°C between the highest and lowest threshold temperatures of 13.3°C and 10.7°C for development of eggs and 4th instar nymphs, respectively.

Development from egg to adult required 419.1 day-degrees (DD). Egg development required an average of 71.8 DD above a base of 13.3°C, and accounted for only about 17.1% of the total thermal development time. The nymphal stage required 341.3 DD, above a base threshold of 11.1°C, and accounted for 81.4% of the total thermal development time. The 2nd instar nymphs completed their development faster than other instars and required only 43.5 DD, accounting for about 12.7% of the total nymphal stage. The 5th instar nymphs required the highest number of thermal units with 96.7 DD, accounting for 28.3% of the total nymphal stage. Thermal units required by the 2nd instar nymphs were about 50% of those required by the 5th instars. The 1st, 3rd, and 4th instars accounted for about 19.2, 15.7, and 21.4%, respectively of the total nymphal development time.

The present study is the first to determine the threshold temperature and thermal constant for development of *N. huttoni*. The threshold temperature for development of nymphs has been determined for *Nysius vinitor* by Kehat & Wyndham (1972a). They reported that the threshold temperature for egg development was 14.5°C and that for nymphs was 15°C. Seventy and 225 DD were required for completing egg and nymphal development, respectively. In

contrast to their results, the threshold temperatures for development of eggs and nymphs were lower in *N. huttoni* than in *N. vinitor*; so the value of the thermal constant for completing nymphal development was greater in *N. huttoni* (341.3 c.f. 225 DD). However, values of the thermal constants for egg development were very similar in *N. huttoni* (71.8 DD) and *N. vinitor* (70 DD).

The threshold temperatures for egg, 1st to 5th instar, nymphal and complete development presented in Table 3-9 for *N. huttoni* are not unlike those determined for the azalea lace bug, *Stephanitis pyrioides* (Scott) (10.2, 12.9, 12.6, 10.2, 12.3, 13.8, 12.2, and 11.2°C, respectively) (Braman *et al.* 1992), and the hawthorn lace bug, *Corythucha cydoniae* (Fitch) (13.3, 16.3, 9.9, 16.4, 17.9, 16.2, 14.9 and 14.3°C, respectively) (Braman & Pendley 1993). A more general developmental threshold temperature of about 11°C was calculated for *Nabis americanoferus* and *N. roseipennis* (Braman 1984).

The threshold temperatures for development of *N. huttoni* were over 10°C and were therefore characteristic of insects in warm regions. Because of this, development may be restricted mainly to hot, summer months. Warm springs followed by hot summers would be expected to favour a rapid build-up of *N. huttoni* populations.

3.3.5 Number of instars in *N. huttoni*

Number of individuals with abnormal instars. In earlier studies (Gurr 1957, Eyles 1960, 1963b), *N. huttoni* was observed to have 5 instars. However, in my studies on the effects of temperature and photoperiod on development, I noticed that the number of nymphal instars was not constant in *N. huttoni* and 4 and 6-instar adults were also produced. The 5-instar adults accounted for over 90% of the population, however. Following my initial observations, detailed records were made to determine the range of variability in instar number and the influence of temperature and photoperiod on this variability. I

also wanted to find out whether there were differences in developmental duration between adults with 4 and 6 instars and adults with 5 instars. The number and percentage of individuals with 4 and 6 instars produced at different temperatures and photoperiods are summarised in Tables 3-10 and 3-11.

Table 3-10. Percentage of adults of *N. huttoni* with 4 and 6 instars at different temperatures.

Temperature (°C)	Total no. adults	Adults with 4 instars		Adults with 6 instars	
		No.	%	No.	%
15	19	2	10.5	0	0.0
20	81	5	6.2	1	1.2
25	47	0	0.0	0	0.0
30	111	7	6.3	2	1.8
35	212	6	2.8	11	5.2
L-T	120	0	0.0	0	0.0

Table 3-11. Percentage of adults of *N. huttoni* with 4 and 6 instars under different photoperiods at 20 and 27.5°C.

Temperature (°C)	Photoperiod (L:D)	Total no. adults	Adults with 4 instars		Adults with 6 instars	
			No.	%	No.	%
20	16:8	71	0	0.0	0	0.0
	12:12	81	5	6.2	1	1.2
	8:16	40	3	7.5	1	2.5
27.5	16:8	104	7	6.7	0	0.0
	12:12	112	4	3.6	2	1.8
	8:16	92	10	10.9	4	4.3

The results in Table 3-10 show that the percentage of 4-instar individuals ranged from 2.8% at 35°C to 10.5% at 15°C; and 6-instar ones ranged from 1.2% at 20°C to 5.2% at 35°C. As temperature increased, the percentage of 4-instar individuals displayed a declining trend, but the percentage of 6-instar individuals displayed an increasing trend. No 4-instar individuals, however, were produced at 25°C and lab temperature, and no 6-instar bugs were produced at 15, 25°C or lab temperature. This indicated that the 4-instar individuals were easily produced at low temperatures, 6-instar ones were easily produced at higher temperatures; and intermediate temperature (e.g., 25°C) and lab temperature were favourable for producing normal 5-instar adults.

The results in Table 3-11 show that an 8-h photoperiod resulted in more 4 and 6-instar adults than other photoperiods. Under a 16-h photoperiod, no 4-instar adults were produced at 20°C and no 6-instar adults at 20 or 27.5°C. The highest percentages of 4 and 6-instar adults both occurred under an 8-h photoperiod at 20°C (7.5% and 2.5%) and 27.5°C (10.9 % and 4.3%). These findings demonstrate that photoperiod affects the number of instars in *N. huttoni*, and that an 8-h short-day photoperiod may induce more “abnormal” instars than a 16-h long-day photoperiod. They also showed there was no directionality in the occurrence of abnormal instars caused by photoperiod. In other words, short-day photoperiod not only decreased but also increased the number of instars. Nevertheless, the percentage of 4-instar adults was higher than that of 6-instar adults at any temperature and photoperiod under which they were produced except in the 35°C treatment. In the latter, the percentage of 6-instar adults (5.2%) was higher than that of 4-instar adults (2.8%). Also, one 3-instar male adult with total developmental duration (egg to adult) of 24 days and one 7-instar male adult with total developmental duration of 19 days were produced at 16-h photoperiod, 27.5°C and 12-h photoperiod, 35°C,

respectively. They were very extreme examples and because only one adult of each kind was found, they are likely to be very rare.

Normally, hemipterans have 5 nymphal instars, but the numbers differ in some families and genera. For example, *Stibaropus formosanus*, *Niphe elongata*, *Liorhyssus hyalinus*, and *Elasmolomus sordidus* can have 6-instar individuals; *Dinorhynchus dybowsky*, *Pentatoma japonica*, *Okeanos guelpartensis*, *Homoeocerus dilatatus*, and *Habrochila chinensis* may produce 4-instar individuals; *Cyrtorrhinus lividipennis* consists mainly of 5-instar individuals but some 4 and 6-instar ones may occur with them. In *Orirus minutus*, 4-instar individuals accounted for the majority of a population investigated by Zhang shi-mei (1985), but a few 3 or 5-instar ones were also found. Because the population of *N. huttoni* I studied was composed mainly of 5-instar individuals (over 90% of individuals), 5 instars are considered to be the norm for this species.

Many factors can cause changes in the numbers of moults in insects. These factors include inadequate nutrition, crowding, inheritance, or external conditions such as temperature and photoperiod. Long (1953) observed that a species of *Plusia* passed through 5, 6, or 7 stadia when reared in isolation, but it apparently passed through only 5 stadia under crowded conditions. Since nymphs were reared in isolation in my study, crowding could not account for the occurrence of 4 or 6-instar individuals of *N. huttoni*.

In my work, all nymphs were supplied with the same food (shepherd's purse) at each temperature and photoperiod treatment, and development of the nymphs was very successful at different temperatures and photoperiods. Inadequate nutrition does not seem to be a reasonable explanation for the occurrence of 4 or 6-instar individuals of *N. huttoni* in my study.

My rearing trials were carried out for only one generation and the number of nymphal instars possessed by the parents was not known before the experiments started. Therefore, no data were obtained that might implicate inheritance as the factor determining instar numbers. Furthermore, most parents that produced 4 or 6-instar offspring also produced 5-instar offspring. Only four pairs of parents (two at 30°C and two at 35°C) produced 3 types of offspring; e.g., 4, 5 and 6-instar individuals. These data suggest that the occurrence of 4 and 6-instar individuals of *N. huttoni* is not genetic.

In some Lepidoptera, there seems to be a relationship between the occurrence of extra instars and the temperature at which the larvae are reared. For example, Leonard (1970) found that he could induce an extra instar in the gypsy moth, *Porthetria dispar* (L), by rearing larvae at temperatures lower than those normally used to rear the moth. With *N. huttoni*, 4 and 6-instar individuals were not induced by rearing nymphs at all temperatures, but temperature is implicated as discussed above.

Photoperiod could be one of the factors inducing 4 and 6-instar individuals of *N. huttoni* and this was demonstrated by the fact that the percentage of 4 and 6-instar individuals was higher under a short-day than long-day photoperiod (Table 3-11). Because 4-instar individuals were produced only at 27.5°C under 16-h photoperiod, it is possible that temperature and photoperiod may interact in some way to produce them.

Moulting hormone and juvenile hormone control the moulting of insects and presumably are implicated in determining the number of moults in *N. huttoni*. Further research is needed to determine the factors that account for the number of instars and their inducement in this species.

Developmental duration of adults with 4 and 6 instars. Developmental duration of insects with 4 and 6 instars are summarised in Tables 3-12, 3-13,

3-14, and 3-15. From Tables 3-12, 3-14 and Fig. 3-7, it can be seen that the 2nd instar was the shortest-lived stage in the development of 4-instar adults at any temperature and photoperiod at which 4-instar individuals were produced. Developmental duration increased gradually from the 2nd to the 5th instar. In 6-instar adults (Tables 3-13 and 3-15), however, the shortest stage was either the 2nd instar (Fig. 3-8 A, B and F), 3rd instar (Fig. 3-8 D) or 4th instar (Fig. 3-8 C and E).

Theoretically, the total developmental time of 4 and 6-instar individuals should be shorter and longer, respectively than that of 5-instar individuals because one instar was lost in the former and gained in the latter. However, developmental times for 4 and 6-instar adults obtained in the lab (Tables 3-16 and 3-17) were both longer and shorter than those of 5-instar adults in the egg stage, nymphal stage and complete life cycle. However, most differences in development time were not significant ($P > 0.05$, T-test). An exception was found at 30°C at which 4-instar nymphs developed significantly ($P < 0.01$) faster than 5-instar ones. On average, the total duration (egg to adult) of 4-instar adults was 3.8 days shorter ($P > 0.05$) at 15°C, 0.7 days longer ($P > 0.05$) at 20°C, 4.1 days shorter ($P < 0.01$) at 30°C, and 1.6 days shorter ($P > 0.05$) at 35°C than that of 5-instar adults at each corresponding temperature. The 6-instar/5-instar and 4-instar/6-instar differences in development duration were not significant ($P > 0.05$).

Photoperiod had no effect on development time of insects with different numbers of instars (5 vs 4-instar, $P > 0.05$) (Table 3-17). Because only one 6-instar adult was produced at 20°C, 12-h photoperiod and 20°C, 8-h photoperiods, statistical comparisons were not made with them. The total developmental duration of the individual under 12-h photoperiod (63 days) was 5.2 and 4.6 days longer than the means for 5 and 4-instar adults (57.8 and 58.4 days) respectively, and the duration for the one grown under 8-h photoperiod (69 days) was 4.0 and 6.3 days longer than the means for 5 and

Table 3-12. Mean developmental duration in days of *N. huttoni* with 4 instars at constant temperatures (range in parentheses).

Temperature (°C)	Sex	No. individuals	Duration					Nymphal duration	Total duration (egg to adult)
			Egg stage	1st instar	2nd instar	3rd instar	4th instar		
15	F	2	24.00 (24-24)	24.00 (20-28)	15.00 (14-16)	17.00 (16-18)	25.00 (24-26)	81.00 (78-84)	105.00 (102-108)
20	F	3	13.67 (13-14)	15.00 (13-17)	6.00 (5-7)	11.00 (8-16)	13.33 (12-15)	45.33 (43-47)	59.00 (57-60)
	M	2	13.00 (13-13)	12.00 (8-16)	9.00 (7-11)	10.00 (8-12)	13.50 (12-15)	44.50 (42-47)	57.50 (55-60)
	F + M	5	13.40 (13-14)	13.80 (8-17)	7.20 (5-11)	10.60 (8-16)	13.40 (12-15)	45.00 (42-47)	58.40 (55-60)
30	F	1	5.00	4.00	3.00	3.00	5.00	20.00	20.00
	M	6	5.00 (5-5)	4.33 (3-6)	2.17 (1-4)	2.83 (2-4)	4.50 (4-5)	13.83 (11-17)	18.83 (16-22)
	F + M	7	5.00 (5-5)	4.29 (3-6)	2.29 (1-4)	2.86 (2-4)	4.57 (4-5)	14.00 (11-17)	19.00 (16-22)
35	F	4	3.00 (3-3)	3.00 (2-4)	2.00 (2-2)	3.25 (2-5)	4.50 (4-5)	12.75 (11-14)	15.75 (14-17)
	M	2	3.50 (3-4)	4.00 (3-5)	2.00 (2-2)	2.50 (2-3)	5.00 (4-6)	13.50 (12-15)	17.00 (16-18)
	F + M	6	3.17 (3-4)	3.33 (1-5)	2.00 (2-2)	3.00 (2-5)	4.67 (4-6)	13.00 (11-15)	16.17 (14-18)

Table 3-13. Mean developmental duration in days of *N. huttoni* with 6 instars at constant temperatures (range in parentheses).

Temperature (°C)	Sex	No. individuals	Duration							Nymphal duration	Total duration (egg to adult)
			Egg stage	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar		
20	M	1	13.00	12.00	2.00	4.00	7.00	9.00	16.00	50.00	63.00
30	F	2	5.00 (5-5)	3.50 (3-4)	2.50 (2-3)	2.00 (1-3)	1.00 (1-1)	3.50 (3-4)	4.00 (4-4)	16.50 (14-19)	21.50 (19-24)
35	F	8	3.00 (3-3)	3.13 (3-4)	1.38 (1-2)	1.25 (1-2)	2.00 (1-3)	2.75 (1-4)	4.13 (3-5)	14.63 (13-16)	17.63 (16-19)
	M	3	3.67 (3-4)	3.33 (2-5)	1.33 (1-2)	2.00 (1-3)	1.67 (1-3)	2.67 (2-3)	4.67 (3-6)	15.67 (12-21)	19.33 (15-25)
	F + M	11	3.18 (3-4)	3.18 (2-5)	1.36 (1-2)	1.45 (1-3)	1.91 (1-3)	2.73 (1-4)	4.27 (3-6)	14.91 (12-21)	18.09 (15-25)

Table 3-14. Mean developmental duration in days of *N. huttoni* with 4 instars under different photoperiods at 20 and 27.5°C (range in parentheses).

Temperature (°C)	Photoperiod (L:D)	Sex	No. individuals	Duration					Nymphal duration	Total duration (egg to adult)
				Egg stage	1st instar	2nd instar	3rd instar	4th instar		
20	12:12	F	3	13.67 (13-14)	15.00 (13-17)	6.00 (5-7)	11.00 (8-16)	13.33 (12-15)	45.33 (43-47)	59.00 (57-60)
		M	2	13.00 (13-13)	12.00 (8-16)	9.00 (7-11)	10.00 (8-12)	13.50 (12-15)	44.50 (42-47)	57.50 (55-60)
		F + M	5	13.40 (13-14)	13.80 (8-17)	7.20 (5-11)	10.60 (8-16)	13.40 (12-15)	45.00 (42-47)	58.40 (55-60)
	8:16	F	2	15.00 (14-16)	18.00 (16-20)	7.00 (7-7)	9.00 (9-9)	14.50 (14-15)	48.50 (46-51)	63.50 (67-60)
		M	1	15.00	17.00	7.00	9.00	13.00	46.00	61.00
		F + M	3	15.00 (14-16)	17.67 (16-20)	7.00 (7-7)	9.00 (9-9)	14.00 (13-15)	47.67 (46-51)	62.67 (60-67)
	16:8	F	2	6.00 (6-6)	6.00 (4-8)	5.50 (5-6)	5.00 (4-6)	6.00 (5-7)	22.50 (21-24)	28.50 (27-30)
		M	5	5.80 (5-7)	7.40 (5-13)	2.80 (1-5)	3.80 (3-5)	5.20 (4-7)	19.20 (14-22)	25.00 (20-28)
		F + M	7	5.86 (5-7)	7.00 (4-13)	3.57 (1-6)	4.14 (3-6)	5.43 (4-7)	20.14 (14-24)	26.00 (20-30)
		F	1	5.00	4.00	6.00	3.00	7.00	20.00	25.00
		M	3	5.67 (5-6)	6.00 (4-8)	3.00 (3-3)	6.00 (4-9)	6.33 (6-7)	21.33 (19-23)	27.00 (25-28)
		F + M	4	5.50 (5-6)	5.50 (4-8)	3.75 (3-6)	5.25 (3-9)	6.50 (6-7)	21.00 (19-23)	26.50 (25-28)
		F	5	6.00 (6-6)	5.80 (3-7)	3.00 (2-6)	5.40 (4-7)	6.40 (6-7)	21.00 (19-23)	26.60 (24-28)
		M	5	6.00 (6-6)	5.20 (3-8)	3.60 (2-5)	4.20 (3-6)	6.80 (5-11)	21.00 (19-23)	25.80 (25-27)
		F + M	10	6.00 (6-6)	5.50 (3-8)	3.30 (2-6)	4.80 (3-7)	6.60 (5-11)	21.00 (19-23)	26.20 (24-28)

Table 3-15. Mean developmental duration in days of *N. huttoni* with 6 instars under different photoperiods at 20 and 27.5°C (range in parentheses).

Temperature (°C)	Photoperiod (L:D)	Sex	No. individuals	Duration							Nymphal duration	Total duration (egg to adult)
				Egg stage	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar		
20	12:12	M	1	13.00	12.00	2.00	4.00	7.00	9.00	16.00	50.00	63.00
	8:16	F	1	15.00	9.00	3.00	7.00	11.00	10.00	14.00	54.00	69.00
27.5	12:12	M	2	5.00 (5-5)	4.00 (3-5)	3.00 (3-3)	2.50 (2-3)	1.00 (1-1)	4.00 (4-4)	7.50 (6-9)	23.00 (22-24)	27.00 (25-29)
	8:16	F	2	6.00 (6-6)	4.00 (4-4)	3.00 (3-3)	20.00 (2-2)	3.50 (2-5)	1.00 (1-1)	5.50 (5-6)	19.00 (17-21)	25.00 (23-27)
		M	2	6.00 (6-6)	4.50 (4-5)	2.50 (2-3)	1.50 (1-2)	2.50 (2-3)	3.50 (3-4)	6.00 (6-6)	20.50 (20-21)	26.50 (26-27)
		F + M	4	6.00 (6-6)	4.25 (4-5)	2.75 (2-3)	1.75 (1-2)	3.00 (2-5)	2.25 (1-4)	5.75 (5-6)	19.75 (17-21)	25.75 (23-27)

Table 3-16. Comparison of mean developmental duration in days of instars of *N. huttoni* with 5, 4 and 6 instars at different temperatures.

Temperature (°C)	Instar	No. individuals	Egg stage	Nymphal stage	Total duration (egg to adult)
15	5	17	24.18	84.65	108.82
	4	2	24.00	81.00	105.00
	5 vs 4	P	> 0.05	> 0.05	> 0.05
20	5	75	13.28	44.45	57.73
	4	5	13.40	45.00	58.40
	5 vs 4	P	> 0.05	> 0.05	> 0.05
30	5	72	5.07	18.07	23.14
	4	7	5.00	14.00	19.00
	6	2	5.00	16.50	21.50
	5 vs 4	P	> 0.05	< 0.01	< 0.01
	5 vs 6	P	> 0.05	> 0.05	> 0.05
	4 vs 6	P	> 0.05	> 0.05	> 0.05
35	5	194	3.27	14.49	17.76
	4	6	3.17	13.00	16.17
	6	11	3.18	14.91	18.09
	5 vs 4	P	> 0.05	> 0.05	> 0.05
	5 vs 6	P	> 0.05	> 0.05	> 0.05
	4 vs 6	P	> 0.05	> 0.05	> 0.05

Females and males combined. Comparisons by T-test.

Table 3-17. Comparison of mean developmental duration in days of instars of *N. huttoni* with 5, 4 and 6 instars under different photoperiods at 20 and 27.5°C.

Temperature (°C)	Photoperiod (L:D)	Instar	No. individuals	Egg stage	Nymphal stage	Total duration (egg to adult)
20	12:12	5	75	13.28	44.45	57.76
		4	5	13.40	45.00	58.40
		6	1	13.00	50.00	63.00
		5 vs 4	P	> 0.05	> 0.05	> 0.05
	8:16	5	36	14.18	50.17	64.97
		4	3	15.00	47.67	62.67
		6	1	15.00	44.00	69.00
		5 vs 4	P	> 0.05	> 0.05	> 0.05
27.5	16:8	5	96	5.95	19.45	25.40
		4	7	5.86	20.14	26.00
		5 vs 4	P	> 0.05	> 0.05	> 0.05
	12:12	5	106	5.66	19.75	25.41
		4	4	5.50	21.00	26.50
		6	2	5.00	23.00	27.00
		5 vs 4	P	> 0.05	> 0.05	> 0.05
		5 vs 6	P	> 0.05	> 0.05	> 0.05
		4 vs 6	P	> 0.05	> 0.05	> 0.05
	8:16	5	78	5.87	20.74	26.62
		4	10	6.00	20.20	26.20
		6	4	6.00	19.75	25.75
		5 vs 4	P	> 0.05	> 0.05	> 0.05
		5 vs 6	P	> 0.05	> 0.05	> 0.05
		4 vs 6	P	> 0.05	> 0.05	> 0.05

Females and males combined. Comparisons by T-test. Because there was only one 6-instar adult produced at 12-h and 8-h photoperiods at 20°C, no T-test was performed between the duration of this individual and others.

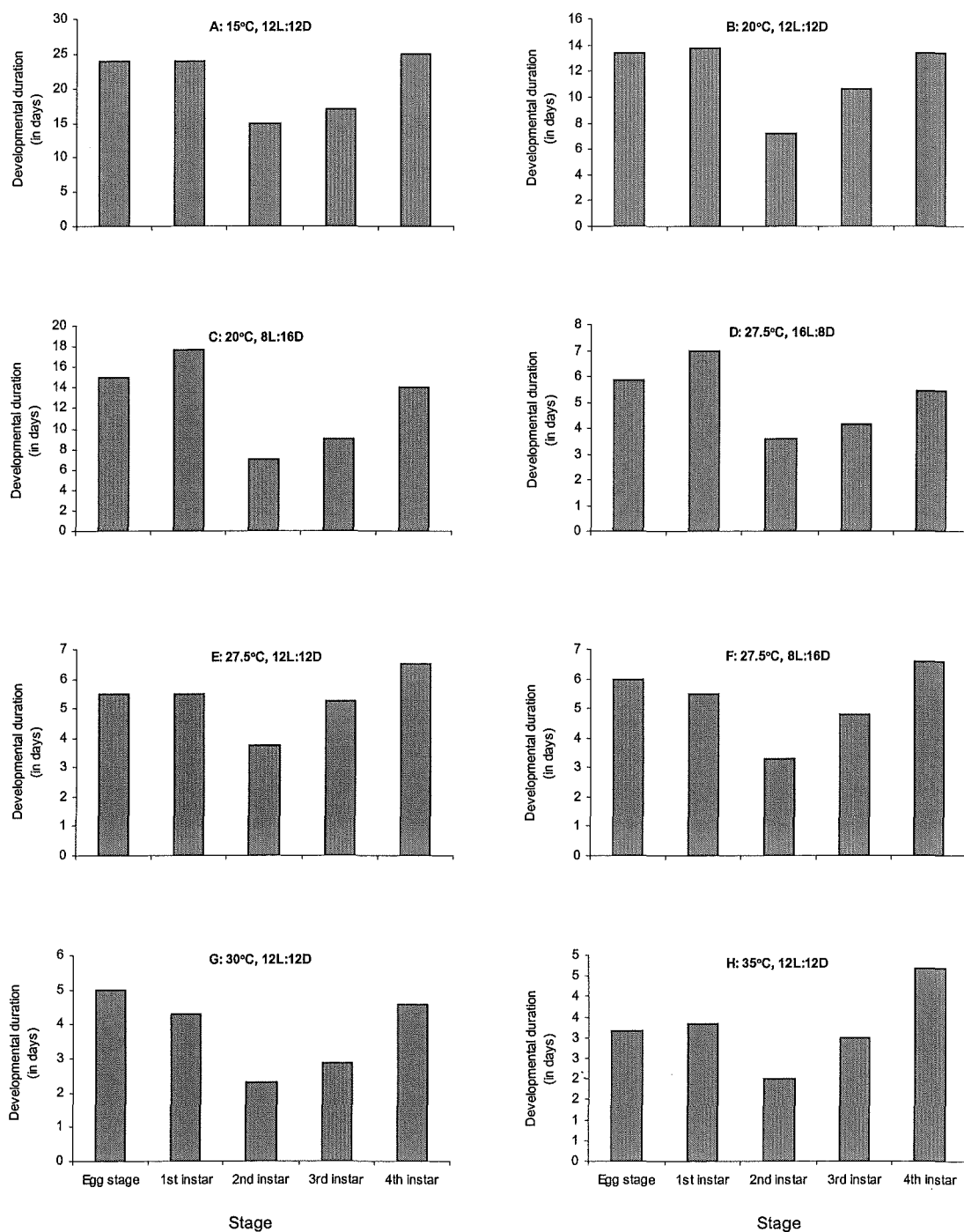


Fig. 3-7. Mean developmental duration in days of 4-instar adults (males and females combined). Standard errors are not shown because numbers of samples are small (see Tables 3-12 and 3-14).

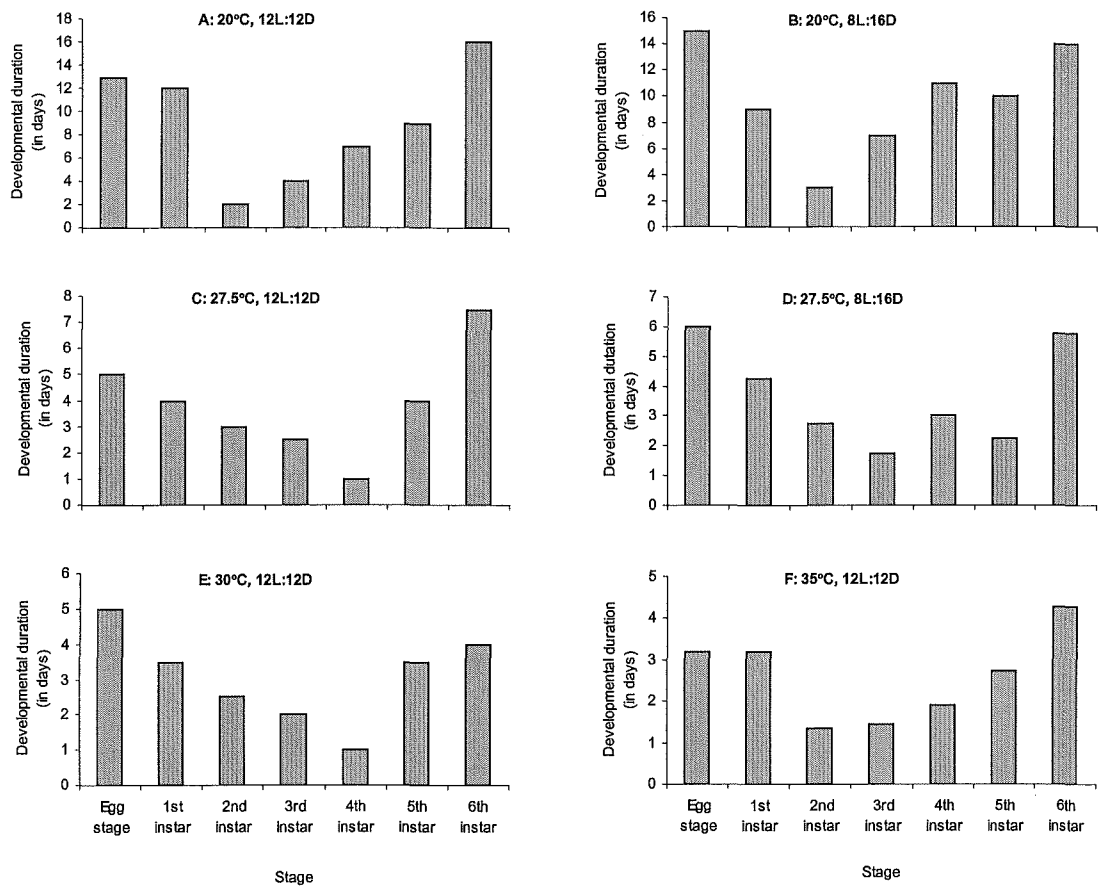


Fig. 3-8. Mean developmental duration in days of 6-instar adults (males and females combined). Standard errors are not shown because numbers of samples are small (see Tables 3-13 and 3-15).

4-instar adults (65 and 62.7 days). At 27.5°C, the 6-instar adults took 1.6 and 0.5 days longer than 5 and 4-instar adults, respectively for development under a 12-h photoperiod, but developmental duration of 6-instar adults under the 8-h photoperiod was 0.9 and 0.5 days shorter than that of 5 and 4-instar adults. None of the above differences was significant ($P > 0.05$).

Overall, it is apparent that instar number had little effect on developmental duration in *N. huttoni*.

3.3.6 Adult longevity

Temperature and longevity. Adult longevity was inversely related to temperature as shown in Table 3-18. Thus, mean adult longevity decreased as temperature increased. The data in Table 3-18 were used to calculate regression equations, and coefficients of determination (r^2) for the relationship between temperature and adult longevity. Results are given in Table 3-19. Fig. 3-9 was prepared by using the data in Table 3-18 to show mean adult longevity at five constant temperatures. The high coefficient of determinations (r^2) of 0.90 for females, 0.94 for males and 0.92 for a combination of females and males indicated that the relationship between temperature and adult longevity was very strong.

The effect of temperature on the longevity of females was highly significant ($P < 0.01$, One-way ANOVA). Mean female longevity varied significantly from a maximum mean of 211.0 days at 15°C to a minimum mean of 11.3 days at 35°C ($P < 0.01$). However, there was no significant difference ($P > 0.05$) in the longevity of females at 30 and 35°C. Mean male longevity, like that of females, was highest (181.3 days) at 15°C and lowest (6.0 days) at 35°C. The effect of temperature was highly significant ($P < 0.01$). No significant difference in longevity was observed at 30 and 35°C ($P > 0.05$).

At lab temperature (mean: 20.3°C; range: 12.5-29.5°C), mean longevity of females and males was 116.1 and 113.7 days, with no significant difference each other ($P > 0.05$, T-test). These results were not significantly different ($P > 0.05$, T-test) from values obtained at 20°C (117.3 days for females and 120.7 days for males).

Maximum longevity of females and males occurred at 15°C (352 and 356 days (about one year) respectively) and minimum longevity occurred at 30 and

Table 3-18. Mean adult longevity in days (\pm S.E.) of *N. huttoni* at different temperatures (range in parentheses).

Sex	No. adults	Temperature (°C)					
		15	20	25	30	35	L-T
F	35	211.0 \pm 17.4 (17-352)	117.3 \pm 10.3 (37-251)	58.2 \pm 8.2 (8-175)	18.4 \pm 1.2 (7-33)	11.3 \pm 1.2 (4-29)	116.1 \pm 18.6 (15-326)
M	35	181.3 \pm 18.9 (15-356)	120.7 \pm 10.4 (32-258)	55.8 \pm 6.0 (8-141)	15.3 \pm 1.5 (3-44)	6.0 \pm 0.7 (4-21)	113.7 \pm 17.8 (9-311)
F + M	70	196.2 \pm 12.9 (15-356)	119.0 \pm 7.3 (32-258)	57.0 \pm 5.0 (8-175)	16.9 \pm 1.0 (3-44)	8.6 \pm 0.8 (4-29)	114.9 \pm 12.8 (9-326)

1. The mean longevity of females and males was not significantly different ($P > 0.05$; T-test) at each temperature except for 35°C ($P < 0.01$).

2. The mean longevity significantly decreased ($P < 0.05$ or < 0.01 ; One-way ANOVA) with increasing of temperature in females, males, and females + males.

3. T-test was performed in comparison of the mean longevity between 20°C and L-T and $P > 0.05$.

Table 3-19. Regression equation, *r* and *r*² values for mean adult longevity of *N. huttoni* in relation to temperature.

Sex	Regression equation (<i>y</i> = <i>a</i> - <i>bX</i>)	<i>r</i>	<i>r</i> ²	<i>P</i>
F	<i>y</i> = 332.4 - 9.966 <i>x</i>	-0.9504	0.9033	< 0.05
M	<i>y</i> = 303.8 - 9.120 <i>x</i>	-0.9705	0.9418	< 0.01
F + M	<i>y</i> = 318.2 - 9.546 <i>x</i>	-0.9617	0.9249	< 0.01

Regression equation *y* = *a* + *bX*, where *y* is mean adult longevity and *x* is temperature. *r* is coefficient of correlation and *r*² is coefficient of determination. When degrees of freedom *V* = 3, *r*_{0.05} = 0.878; and *r*_{0.01} = 0.959. All *r* values > *r*_{0.05} = 0.878 or *r*_{0.01} = 0.959, so *P* < 0.05 or 0.01.

35°C (3-4 days for both sexes). These data indicate that high temperatures (≥ 30°C) are not conducive to longevity of adults. Long survival at low temperatures is highly advantageous, since overwintering adults form the basis of populations the next spring.

Mean longevity was not significantly different (*P* > 0.05, T-test) between females and males except at 35°C, when females lived longer than males (*P* < 0.01). The difference in longevity among different individuals within the same sex at the same temperature was great, however. For example, at 15°C, the difference between the maximum and minimum was 335 days in females and 341 days in males; at 35°C, the difference for females and males was 25 days and 16 days, respectively. Similarly, a large difference was found at lab temperature at which minimum and maximum longevity were 15 and 326 days in females (a difference of 321 days) and 9 and 311 days in males (a difference of 302 days).

N. huttoni lived for about four months at fluctuating temperatures averaging close to 20°C, and at a constant temperature of 20°C, while it could live for at

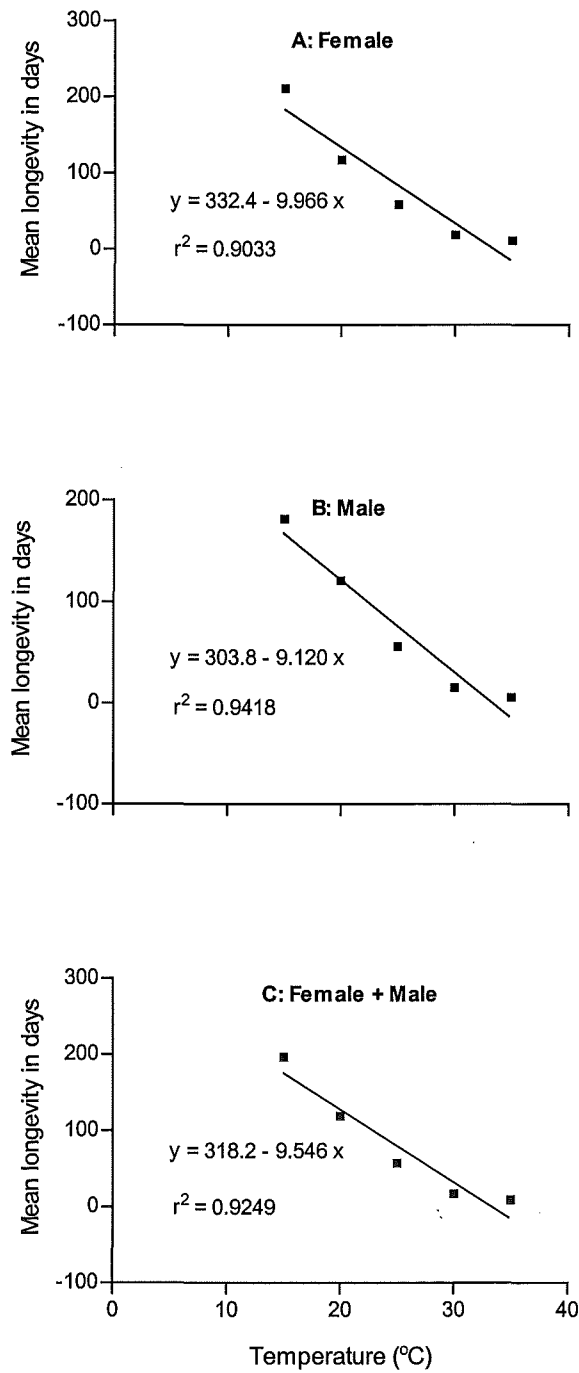


Fig. 3-9. Relationships between mean longevity and temperature for male and female adults.

least six months at 15°C. This was not unexpected, since adults of the third generation (see Chapter 4) must live from early or mid-February when adults emerged before winter until later October or early November when adults disappeared after winter.

Survival periods of adult females of insects can be significantly longer or shorter than those of adult males, or the same length (Leigh 1963, Khattat & Stewart 1977, Kehat & Wyndham 1972a, and John *et al.* 1988, 1990). In *N. huttoni*, there was no significant difference ($P > 0.05$, T-test) in longevity between females and males at temperatures below 35°C, but at high temperature (35°C), longevity of females was significantly greater than that of males ($P < 0.01$). This demonstrated that adult females could withstand higher temperatures ($\geq 35^\circ\text{C}$) better than males.

In order to confirm that female adults had greater endurance of high temperatures ($\geq 35^\circ\text{C}$) than males, a supplementary test was conducted at three constant high temperatures of 36, 38 and $40 \pm 0.5^\circ\text{C}$ on 9-11 December 1997. Fifty male and 50 female adults of the first generation collected from the field were held at each temperature. Ten adult males and 10 females were placed in each of five glass tubes. Plenty of food in the form of shepherd's purse was supplied during the test. Twenty-four hours later, the number of dead adults was counted. The results (Table 3-20) showed that mortality of males was higher than that of females at each temperature. During the summer, ground temperatures can reach up to 40-55°C and under such conditions males would be expected to die earlier than females. The sex ratio and its variation in the field suggests this may well happen (see Chapter 4). It is probable, therefore, that adult longevity of male and female *N. huttoni* does not differ at temperatures below 30°C, but females live longer than males at temperatures above 30°C.

Table 3-20. Mortality of adults of *N. huttoni* at high temperatures in laboratory trials. M = male, F = female.

Temperature (°C)	No. adults tested		No. adults died in 24 hours		Mortality (%)	
	F	M	F	M	F	M
36	50	50	15	21	30	42
38	50	50	15	26	30	52
40	50	50	27	35	54	70

Test was carried out between 9 and 11 December 1997. Adults tested were those of the first generation collected from the field.

These findings contrast sharply with those of Kehat & Wyndham (1972a) who reported that males of *Nysius vinitor* lived longer than females. In *Stephanitis pyrioides* (Neal & Douglass 1988) and *Corythucha cydoniae* (Neal & Douglass 1990), males lived longer than females, while in *Lygus hesperus* (Leigh 1963), *Geocoris punctipes* (Robert *et al.* 1967) and *Ooencyrtus papilionis* (Rahim *et al.* 1991), females outlived males.

Photoperiod and longevity. The effect of photoperiod on adult longevity at 20°C is shown in Table 3-21. Photoperiod had a significant effect on adult longevity ($P < 0.05$, One-way ANOVA). Both males and females lived longer under an 8-h short-day photoperiod than under a 16-h long-day photoperiod. The mean longevity of female adults was 85.9, 122.2, and 167.5 days under 16-h, 12-h, and 8-h photoperiods, respectively. Corresponding mean longevity for male cohorts was 74.7, 118.0, and 151.5 days under each photoperiod. Maximum longevity was 258 days for females and males under an 8-h photoperiod. The difference in mean longevity of males, females and both sexes combined was not significant ($P > 0.05$, One-way ANOVA) between the 16-h and 12-h, and the 12-h and 8-h photoperiods, but significant ($P < 0.01$) between the 16-h and 8-h photoperiods. No difference was found in the longevity of males and females held at particular photoperiods ($P > 0.05$, T-test).

Table 3-21. Mean adult longevity in days (\pm S.E.) of *N. huttoni* at different photoperiods and 20°C (range in parentheses).

Sex	No. adults	Photoperiod (L:D)		
		16:8	12:12	8:16
F	10	85.9 \pm 4.3 (63-107) b	122.2 \pm 17.7 (73-209) ab	167.5 \pm 19.2 (88-258) a
M	10	74.7 \pm 5.0 (48-88) b	118.0 \pm 20.3 (42-255) ab	151.5 \pm 25.0 (14-258) a
F + M	20	80.3 \pm 3.4 (48-107) b	120.1 \pm 13.1 (42-255) ab	159.5 \pm 15.5 (14-258) a

1. Means followed by the same letter in a line were not significantly different at the 5% significance level (One-way ANOVA).

2. Mean longevity of females and males was not significantly different ($P > 0.05$; T-test) at each photoperiod.

The data presented here indicate that an 8-h short-day photoperiod favours adult longevity of *N. huttoni*. These findings are consistent with field observations. Adults of the third generation, as mentioned above, had the greatest longevity in the field in contrast to those of the first and second generations. Thus, when adults of the third generation initially emerged in the field (early February 1997 and 1998 and early March 1995 and 1999), day-length was shortening (14 h, 5 min sunlight on 10 February, and 13 h, 4 min on 2 March); and when adults of the third generation disappeared from the field, it was later October (1998) or early November (1996 and 1997) when days were lengthening, gradually (13 h, 51 min sunlit time on 28 October, and 14 h, 18 min on 7 November). However, long days in the field are linked with high temperatures in spring and summer, and short days are linked with lower temperatures in autumn and winter. Low temperature combined with a short photoperiod signals the onset of winter, and a period of adult longevity.

Weseloh (1986) also found that females of *Ooencyrtus kuvanae* reared at 10-h and 12-h photoperiods lived longer than those held at 14-h or 16-h photoperiods. The results presented here indicate that short daylength is one of the factors resulting in long adult longevity of *N. huttoni*.

3.3.7 Fecundity and oviposition activities

Temperature and fecundity. At each temperature, 35 female adults were tested for oviposition activity and adult longevity (Table 3-22).

Number of the female adults that laid eggs. All the tested females (100%) laid eggs at 20°C, whereas the number of females that laid eggs was lowest at 15°C (65.7%). The proportions at other temperatures ranged from 77.1% to 97.1%. These data reveal that not all females laid eggs during their lives, but the reason for this is unknown. However, it may be related to temperature since the temperature range required for oocyte development is generally narrower than that required for egg and nymphal development. A temperature of 15°C may be very close to the threshold temperature for oocyte development in *N. huttoni*.

I also found that females that laid eggs lived longer than those that did not. Thus, of the 35 females tested at 15°C, mean longevity of the 23 that laid eggs was 264.0 days. However, the 12 females that did not lay eggs had a mean longevity of only 109.4 days. The former was significantly longer ($P < 0.01$, T-test). The mean longevity of 109.4 days for female adults that did not lay eggs was shorter than the shortest pre-oviposition periods of 131 days for females that laid eggs. This indicated that at 15°C, those females that did not lay eggs died within the pre-oviposition period. At 25°C, the mean longevity of 27 females that laid eggs was 70.1 days and that of females that did not lay eggs was only 18.1 days ($P < 0.01$). Similarly, 31 females that laid eggs at 30°C had a mean longevity of 19.6 days, significantly longer ($P < 0.01$) than that of 4 adults that survived for 9.3 days on average, but did not lay any eggs.

Pre-oviposition periods. Pre-oviposition periods of female adults decreased as temperature increased, except at 25°C. At 15°C, females had the longest pre-

Table 3-22. Periods of ovipositional activity and oviposition rate (\pm S.E.) of female *N. huttoni* at different temperatures (range in parentheses).

Temperature (°C)	No.females laid eggs/35	Pre-oviposition period (days)	No.eggs laid per female	Relative oviposition period (days)	Absolute oviposition period (days)	Relative daily mean eggs laid per female	Absolute daily mean eggs laid per female	Min eggs laid per female per day	Max eggs laid per female per day	Max no.days successive laying	Min interval time without laying (days)	Max interval time without laying (days)	Post-oviposition period (days)
15	23 (65.7%)	155.8 \pm 4.91 (131-224) a	37.8 \pm 6.10 (3-98) b	87.8 \pm 11.80 (1-82) a	17.9 \pm 2.49 (1-46) b	0.6 \pm 0.12 (0.1-3.0) c	2.1 \pm 0.12 (1.1-3.3) c	1.1 \pm 0.09 (1-3) b	4.8 \pm 0.30 (2-8) d	3.2 \pm 0.52 (0-11) c	1.3 \pm 0.22 (0-5) a	32.1 \pm 6.61 (0-101) a	20.3 \pm 3.47 (0-52) a
20	35 (100.0%)	7.7 \pm 0.12 (7-9) c	292.9 \pm 17.53 (77-484) a	99.8 \pm 9.23 (26-235) a	70.9 \pm 4.49 (19-124) a	3.2 \pm 0.14 (1.9-4.6) b	4.2 \pm 0.09 (3.0-5.0) b	1.0 \pm 0.00 (1-1) b	11.1 \pm 0.38 (7-18) b	21.4 \pm 1.35 (8-42) a	1.0 \pm 0.00 (1-1) a	15.8 \pm 3.57 (1-59) b	9.9 \pm 4.30 (0-131) ab
25	27 (77.1%)	38.7 \pm 6.20 (4-99) b	59.6 \pm 10.88 (1-235) b	26.7 \pm 4.23 (1-82) b	17.2 \pm 2.82 (1-61) b	2.5 \pm 0.22 (0.3-4.4) b	3.2 \pm 0.19 (1.0-4.6) bc	1.1 \pm 0.05 (1-2) b	7.1 \pm 0.58 (1-15) cd	8.1 \pm 0.92 (0-18) b	0.8 \pm 0.11 (0-2) a	4.4 \pm 1.13 (0-25) bc	4.7 \pm 1.07 (0-24) b
30	31 (88.6%)	5.5 \pm 0.36 (3-10) c	45.1 \pm 6.11 (2-132) b	13.2 \pm 1.25 (2-27) b	10.4 \pm 1.08 (2-22) b	3.2 \pm 0.29 (0.6-6.7) b	3.8 \pm 0.28 (1.0-6.7) b	1.3 \pm 0.09 (1-2) b	7.8 \pm 0.70 (1-16) c	7.5 \pm 0.79 (2-18) b	1.2 \pm 0.22 (0-5) a	1.8 \pm 0.31 (0-6) c	1.1 \pm 0.26 (0-6) b
35	34 (97.1%)	1.5 \pm 0.09 (1-2) c	73.4 \pm 8.34 (11-176) b	8.9 \pm 1.10 (2-26) b	8.5 \pm 1.01 (2-22) b	8.8 \pm 0.47 (5.5-15.7) a	9.0 \pm 0.46 (5.5-15.7) a	3.7 \pm 0.55 (1-14) a	15.4 \pm 0.72 (6-22) a	7.4 \pm 0.87 (2-17) b	0.2 \pm 0.07 (0-1) b	0.3 \pm 0.11 (0-3) c	1.1 \pm 0.43 (0-15) b
L-T	34 (97.1%)	38.8 \pm 12.20 (8-295)	71.5 \pm 10.77 (1-207)	74.2 \pm 15.57 (1-304)	23.6 \pm 3.51 (1-69)	1.8 \pm 0.20 (0.1-4.1)	2.9 \pm 0.17 (1.0-4.7)	1.1 \pm 0.05 (1-2)	7.1 \pm 0.64 (1-15)	8.2 \pm 1.01 (0-24)	1.1 \pm 0.22 (0-7)	32.2 \pm 10.3 (0-272)	6.0 \pm 1.15 (0-29)

One-way ANOVA was used in comparison of means among constant temperatures. Means followed by the same letter in a column were not significantly different at the 5% significance level.

oviposition period: mean 155.8 days, minimum 131 days, maximum 224 days. The shortest pre-oviposition period (mean 1.5 days; range 1-2 days) was obtained at 35°C. At 25°C, the mean pre-oviposition period theoretically should be shorter than that (7.7 d) of females at 20°C and longer than that (5.5 d) of females at 30°C. However, the observed value was 38.7 days. The probable reason for this was that adults tested at 25°C had emerged from nymphs of the second generation collected from the field in mid-January 1996. During that time, host plants at the research site dried off because of hot weather. Therefore, the nymphs might not have been well fed and this may have affected their oviposition activity. A similar result was obtained at lab temperature, for which the 35 females tested for oviposition activity were those that emerged from nymphs that reared in the laboratory and fed on a single host plant, shepherd's purse. Female adults kept at both 25°C and lab temperature therefore had nearly the same pre-oviposition periods (38.7 and 38.8 days). These data reveal that nutritional state in the nymphal stage may affect the length of the pre-oviposition period and oviposition of adults.

My experiments also showed that large difference in pre-oviposition periods occurred at the same temperature among different individuals. For example, minimum and maximum pre-oviposition periods were 131 and 224 days, respectively at 15°C, 4 and 99 days at 25°C, and 8 and 295 days at lab temperature. The reason for this is unknown.

Mean total number of eggs (MNE). MNE significantly increased between 15 and 20°C and was significantly reduced between 20°C and higher temperatures ($P < 0.01$). The highest MNE occurred at 20°C (292.9/♀) and the lowest at 15°C (37.8/♀). A significant difference in the total number of eggs per female occurred between 20°C and each of other temperatures ($P < 0.01$), but no difference was found among the other 4 constant temperatures ($P > 0.05$). At lab temperature, the MNE was 71.5/♀. A big difference in the number of

eggs per female existed among different individuals at each temperature. A female (except for eggless ones) was found to lay a minimum of one or two eggs (at 25, 30°C and lab temperature) and a maximum of 484 eggs (at 20°C). Females that laid one or two eggs did not attain the average longevity of members of their temperature group. For example, at lab temperature, two females that laid one egg each both lived for 18 days, whereas mean longevity was 116.1 days. The adults that produced one egg at 25°C and two eggs at 30°C lived for 16 days and 14 days, respectively, and therefore did not reach the mean ages of 58.2 and 18.4 days obtained at these two temperatures.

Relative and absolute oviposition periods. Relative oviposition periods (the maximum time over which oviposition occurred) were significantly longer ($P < 0.05$) at low temperatures (15-20°C) than at high temperatures (25-35°C). The longest relative oviposition period (mean 99.8 days) was obtained at 20°C and the shortest (mean 8.9 days) at 35°C. Difference between 15 and 20°C and between 25 and 35°C were not significant ($P > 0.05$). Absolute oviposition periods (the actual number of days on which oviposition occurred within the relative oviposition period) and the total number of eggs laid per female at various constant temperatures were associated. Largest total egg production (292.9 eggs/♀) occurred over the longest oviposition period (70.9 days) which was at 20°C. Differences in egg production were significant between 20°C and other temperatures ($P < 0.05$).

Daily egg laying per female. Temperature influenced the mean daily oviposition rate of females. Relative and absolute mean maximum numbers of eggs laid daily per female occurred at 35°C (8.8/♀ and 9.0/♀ eggs) and minimum numbers occurred at 15°C (0.6/♀ and 2.1/♀ eggs). Significant differences ($P < 0.05$) were found in the number of eggs laid daily between 15 or 35 °C and other temperatures except 25°C, at which the absolute daily mean number of eggs was not significantly different from that at 15°C ($P >$

0.05). No significant difference ($P > 0.05$) in numbers of eggs laid was found within the range 20-30°C. Greatest daily egg laying at the highest temperature (35°C) again indicates the species is well adapted to warm habitats.

Mean minimum and maximum daily eggs per female. Minimum and maximum numbers of eggs laid per female per day were recorded at each temperature. At 35°C, females laid a mean minimum of 3.7 eggs per day, whereas at other temperatures, the minimum was 1.0–1.3 eggs per day. The mean maximum number of eggs per female ranged from 15.4 eggs per day at 35°C to 4.8 eggs at 15°C.

Maximum number of days of successive laying and interval time without laying. The maximum number of days of successive egg laying and the length of the interval when no eggs were laid differed at different temperatures. The highest number of days of successive egg laying was observed at 20°C (mean 21.4 days; range 8-42 days) and the lowest was at 15°C (mean 3.2 days; range 0-11 days). The difference between the two was significant ($P < 0.05$). The successive laying times of 8.1 days at 25°C, 7.5 days at 30°C and 7.4 days at 35°C were not significantly different from each other ($P > 0.05$), but they were significantly different from values at 15 and 20°C ($P < 0.05$).

The mean interval time without any eggs being laid was longer at low temperature than at high temperature and decreased as the temperature increased. At 15°C, the mean minimum and maximum intervals without laying eggs were 1.3 days (range 0-5 days) and 32.1 days (0-101 days), respectively, whereas at 35°C, the mean minimum and maximum intervals were 0.2 day (0-1 day) and 0.3 day (0-3 days), respectively. The mean minimum and maximum intervals recorded at fluctuating temperature were 1.1 days (0-7 days) and 32.2 days (0-272 days). These data indicate that at temperatures below 25°C and at fluctuating temperature, the range of variation

in the maximum interval without laying eggs was greater. Above 30°C, the maximum interval was no more than 6 days.

Post-oviposition period. Mean length of the post-oviposition period decreased with increasing temperature. It was longest at 15°C (20.3 days) and shortest at 30 and 35°C (1.1 days). Some females died on the day they laid their last egg, while some had a long post-oviposition period. The longest post-oviposition period recorded was 131 days at 20°C. The mean post-oviposition period at fluctuating temperature was 6 days with a range of 0-29 days. These data showed that there were substantial differences in post-oviposition period length among individuals even at the same temperature.

Photoperiod and fecundity. Effects of photoperiod on fecundity are summarised in Table 3-23. In this experiment, ten adults were tested for oviposition and adult longevity and One-way ANOVA was used to compare means among different photoperiods. Photoperiod did not have a significant influence ($P > 0.05$) on the length of the pre-oviposition period in *N. huttoni* even though the pre-oviposition period under an 8-h photoperiod was 15.6 days, which was almost twice as long as those under 16-h and 12-h photoperiods. However, the 8-h value was strongly influenced by one female that had a long pre-oviposition period of 92 days, whereas the pre-oviposition period of the other 9 females was 7-8 days.

The highest mean number of eggs (325.3) per female was obtained under the 12-h photoperiod, but it was not significantly different ($P > 0.05$) from that under the 16-h photoperiod (263.8). However, it was significantly different ($P < 0.01$) from that under the 8-h photoperiod (114.0). The absolute oviposition period itself had a mean of 31.9 days per female under the 8-h photoperiod, which was about half that under the 16-h photoperiod (61.6 days) and less than half that under the 12-h photoperiod (75.9 days). The difference was highly significant ($P < 0.01$). Females laid an average of 3.4 eggs per day

Table 3-23. Periods of ovipositional activity and oviposition rate (\pm S.E.) of *N. huttoni* under different photoperiods at 20°C (range in parentheses).

Photoperiod (L:D)	No.females tested	Pre-oviposition period (days)	No.eggs laid per female	Relative oviposition period (days)	Absolute oviposition period (days)	Relative daily mean eggs laid per female	Absolute daily mean eggs laid per female	Min eggs laid per female per day	Max eggs laid per female per day	Max no.days successive laying	Min interval time without laying (days)	Max interval time without laying (days)	Post-oviposition period (days)
16:8	10	8.0 \pm 0.30 (7-9) a	263.8 \pm 24.52 (144-346) a	75.9 \pm 4.56 (52-98) a	61.6 \pm 4.24 (41-81) a	3.4 \pm 0.20 (2.7-4.4) a	4.2 \pm 0.19 (3.5-5.0) a	1.0 \pm 0.00 (1-1) a	10.3 \pm 0.63 (7-13) a	16.9 \pm 1.42 (13-25) a	1.0 \pm 0.00 (1-1) a	3.5 \pm 0.47 (1-6) b	2.0 \pm 0.47 (1-6) b
12:12	10	8.0 \pm 0.15 (7-9) a	325.3 \pm 28.71 (179-484) a	106.0 \pm 16.74 (64-196) a	75.9 \pm 6.72 (55-115) a	3.4 \pm 0.28 (2.0-4.6) a	4.3 \pm 0.21 (3.0-5.0) a	1.0 \pm 0.00 (1-1) a	12.2 \pm 1.01 (7-18) a	22.5 \pm 1.73 (14-31) a	1.0 \pm 0.00 (1-1) a	15.8 \pm 6.96 (1-58) b	8.2 \pm 6.13 (0-63) b
8:16	10	15.6 \pm 8.49 (7-92) a	114.0 \pm 22.40 (2-211) b	109.5 \pm 23.91 (2-244) a	31.9 \pm 5.38 (2-59) b	1.5 \pm 0.38 (0.4-4.1) b	3.4 \pm 0.43 (1.0-5.9) a	1.0 \pm 0.00 (1-1) a	8.9 \pm 1.41 (1-17) a	10.1 \pm 2.15 (2-26) b	1.1 \pm 0.18 (0-2) a	49.1 \pm 13.73 (0-117) a	42.4 \pm 24.55 (1-236) a

One-way ANOVA was used in comparison of means among different photoperiods. Means followed by the same letter in a column were not significantly different at the 5% significance level.

under both the 16-h and 12-h photoperiods, but only 1.5 eggs per female under the 8-h photoperiod ($P < 0.01$). These results indicate that short day length can bring about a decrease in the total number of eggs laid per female, a decrease in the length of the absolute oviposition period and a decrease in the mean number of eggs laid per female per day.

Photoperiodic effects on insects are usually associated with effects on diapause induction. Under an 8-h short-day photoperiod, the total number of eggs and the number laid per day were lowest, whereas the mean interval time without laying eggs, and the post-oviposition period, were longest. These factors are closely linked with diapause in this species and will be discussed in more detail in Chapter 8.

CHAPTER FOUR

**Life history and some aspects
of biology and ecology of
*N. huttoni***

Chapter 4

Life history and some aspects of biology and ecology of *N. huttoni*

4.1 Introduction

Insects are extraordinarily adaptable animals. They have evolved to live successfully in diverse environments on earth, including ice-capped poles, tropical rain forests, cold rivers, swamps and deserts. Their small size and ability of flight permit them to escape from enemies and disperse to new environments (Caltagirone 1999). In addition, due to their small size, insects require only small amounts of food to complete development and can exist in very small spaces. Environments of insects involve many biotic factors such as food sources, natural enemies, and inter- or/and intra-specific competition, and abiotic factors such as temperature, humidity, light and wind (Yazdani & Agarwal 1997). These factors vary seasonally and exert direct or indirect influence on growth, development, reproduction, dormancy and migration. Most insects have evolved the ability to adapt to these variations (Tauber *et al* 1986). Successful management of a pest requires a better understanding of effects of these factors on the pest.

Since insects grow and develop within populations, in studies of their biology and ecology, it is better to consider these populations as well as individuals, especially within the context of an agroecosystem. Insect populations have attributes such as density, cohort and age structure, birth and death dates (Yazdani & Agarwal 1997). Insect population dynamics are affected by many factors. These could be genetic factors, food supply, weather, and farming practices.

Accurate prediction of occurrence and duration of certain stages of an insect in the field has many applications in efforts of understanding the insect's

population dynamics (Fielding & Ruesink 1988). IPM programs are based on an awareness of the timing of seasonal events to enable one to predict the occurrence of particular life stages of a pest (Tauber *et al* 1986). Successful IPM requires a thorough knowledge of the biology of the pest insects and better understanding of both population dynamics and effects of environmental factors (Gullan & Cranston 2000).

N. huttoni was reported to overwinter in the adult stage (Gurr 1952). Its populations can survive and develop on a wide range of plant species, and most hosts are common weed species of New Zealand pastures and waste grasslands (Myers 1921, 1926; Eyles 1965a, 1965b; Gurr 1952, 1957; Woodward 1954). Due to its widespread distribution, occurrence, and noted importance to wheat and other cultivated plants, some aspects of its biology and ecology have been investigated (Myers 1926; Eyles 1965b; Gurr 1952, 1957; and Farrell & Stufkens 1993). However, many aspects of its biology and ecology remain poorly understood.

Eyles (1963a, 1963b) has reported on the incubation period, nymphal development, fecundity, and oviposition rhythms of *N. huttoni* reared in a greenhouse, but the seasonal biology of copulation and oviposition in the field have not been reported. Ferro (1976) reported that a single copulation can fertilise a female for life, but no experimental evidence was found in the literature to support this conclusion. Furthermore, in my review of literature on *N. huttoni*, information on patterns of seasonal development and occurrence, sex ratio and its seasonal variation, and population variation in the field is lacking.

Reports of the number of generations per year for this species are variable. Myers (1926) suggested that more than one generation per year could occur. Two generations were reported by Gurr (1952) and Farrell & Stufkens (1993), and three or four generations by Eyles (1963b, 1965b). To examine whether

variation occurs in the number of generations per year, my study included field observations and sampling as well as laboratory rearing. I obtained data on the following: 1) overwintering habitats and time entering overwintering and spring emergence; 2) the number of generations occurring per year in Canterbury; 3) the occurrence and duration of development of each generation in the field; 4) copulation and oviposition activities of the adults in the field; 5) sex ratio and its seasonal variation; and 6) population trends in the field.

4.2 Materials and methods

4.2.1 Overwintering survey

Overwintering surveys were made in the field during 1996-2000. In autumn and spring, very frequent visits to the field (about once every 3 days) were made. During winter, visits were made weekly. The habits, host plants, overwintering stage, and times of entering overwintering sites in autumn and emergence in spring were recorded.

4.2.2 Number of generations per year

To determine the number of generations completed by *N. huttoni*, two methods were used: 1) direct field observations; 2) field sampling.

Direct field observations. Field observations were made at the research site at 5-day intervals from August 1995 to December 1999. Developmental duration and periods of occurrence of each generation were recorded. Here it is necessary to point out that developmental duration and period of occurrence are different. Developmental duration refers to the period of time required by eggs, or nymphs to complete their development; whereas the period of occurrence refers to a period of time during which eggs, nymphs, and adults of a cohort occur in the field. That is, it is the time from appearance to

disappearance of a stage in the field. The developmental duration of a life stage is shorter than the period of occurrence of that stage. The developmental periods of all stages, including all individual variation in duration, sums to the period of occurrence of a cohort.

As *N. huttoni* lays eggs in the soil, it was very difficult to determine the developmental duration and period of occurrence of eggs in the field. The developmental duration of eggs belonging to each generation was therefore estimated according to the emergence time of the adults of the last generation and the time of initial appearance of the newly emerged 1st instar nymphs of the next generation. The period of occurrence of eggs of each generation was estimated according to the period of occurrence of the adults of the last generation. Female adults were dissected to examine eggs in 1997-1998 and 1998-1999 so that developmental duration and occurrence of eggs could be determined more accurately.

Duration of nymphal development of each cohort in the field was determined by noting the time from initial appearance of newly hatched 1st instar nymphs to the initial appearance of newly emerged adults. The period from initial appearance of newly hatched 1st instar nymphs to emergence as adults of 5th instar nymphs of the same cohort was determined as the occurrence period of nymphs for this generation. Since continual oviposition by females results in continual hatching of nymphs, there are large differences in ages among individual nymphs of the same cohort. Thus, it is impossible to estimate the duration and period of occurrence of each instar from field observations. Therefore, only total duration of development, and total period of occurrence from first to fifth instar were estimated.

Field sampling. Bugs were collected at 10-day intervals from five randomly placed 0.25 m² (0.5 m x 0.5 m) quadrats on the ground at the research site, using a domestic vacuum cleaner. Collections were made for two years, 1995-

1996 and 1996-1997. In 1995, collections began on 5 September and were made on the 5th, 15th, and 25th of each month until 5 June 1996. The same schedule was repeated beginning on 5 September 1996 and ending on 5 April 1997. Field-collected insects from each quadrat were placed in ice cream boxes and returned to the laboratory where adults and nymphs were separated from each other and counted. Because it was impossible to differentiate all nymphal stages, they were recorded simply as nymphs (Tables 4-1 and 4-2). Numbers of adults and nymphs were recorded for each area and the mean for all 5 areas was calculated. Numbers of generations were determined based on seasonal variation in the proportions of adults and nymphs. These samples were also used to determine seasonal variation of the field population.

4.2.3 Seasonal copulation activities in the field

N. huttoni has been found to overwinter as adults. However, it is unknown whether overwintering adults mate before or after winter, or at both times. Mating times for other generations and seasonal patterns of oviposition are not known, either. The purpose of my investigation was to clarify this. Copulation activities were observed and recorded in the field at 5-day intervals from August 1995 to May 1999. Copulation was scored on a scale of 0-3 (0 = no copulation seen in the field; 1 = initial period of copulation indicated by a few adults copulating; 2 = many adults copulating; and 3 = the copulation peak during which very many adults were copulating).

4.2.4 Seasonal oviposition activities in the field

To clarify the seasonal oviposition pattern of *N. huttoni* in the field, female adults were collected in 1997-1998 and 1998-1999 and dissected to determine their state of reproductive maturity by examining eggs. Collections starting on 5 September and ending on 15 April each year were made at 10-day intervals

and carried out on the 5th, 15th, and 25th of each month. Thirty females were dissected on each sampling date, and the stage of ovarian development was recorded. Females that had either laid eggs, or had mature or developing eggs were classed as reproductive and thus were nondiapausing; females that had no mature or developing eggs present were classed as nonreproductive and were assumed to be in pre-ovipositional state (in females of the first and second generation) or to be in diapause (females of the third generation). This work was also used to confirm the occurrence of reproductive diapause in adults of the third generation.

4.2.5 Sex ratio

Sex ratio of *N. huttoni* was investigated in three ways.

1. Fifth instar nymphs were collected from the field and kept in the laboratory to emerge as adults. Adults that emerged were sexed. These investigations were made from 14 November 1997 to 11 February 1998 during which 8 collections were made.
2. Sex ratios of adults produced at different temperatures and photoperiods in the lab were recorded (see Chapter 3).
3. Adults were collected from the field at 10-day intervals on the 5th, 15th, and 25th of each month during 1995-1999. Adults were sorted in the laboratory and numbers of females and males were recorded. These collections were made as the seasons progressed, so the results would reflect any seasonal changes in sex ratio.

The sex ratio was calculated and expressed as ♀:♂ = 1:x according to the proportion of females to males of the total number of measured adults for each

sample or total adult population. Departures from an expected 1:1 (♀:♂) ratio were examined with a chi-squared test.

4.2.6 One copulation fertilises a female for life

Ferro (1976) reported that a single copulation fertilises a female for life. No report, however, was found that provided evidence in support of this statement. I therefore carried out an experiment to test this proposition and discover over what period a female could remain fertile after one mating. Fifth instar nymphs were collected from the field on 18 December 1997. After they emerged as adults in the laboratory, 14 pairs of adults (one male and one female in each pair) were reared as described in Chapter 3. When each pair had completed one copulation, the male was removed. Eggs laid every 5 days after the first day of egg laying were separated to test their hatching ability. The experiments were conducted until all females died. The rearing of female adults and the hatching tests were conducted at 25°C with 12-h photoperiod. Pre- and post-oviposition periods, oviposition periods, adult longevity and hatching of eggs were recorded.

4.2.7 Parthenogenesis

Buxton (1930), who worked with the blood-sucking bug, *Rhodnius prolixus*, tested 307 eggs produced by 10 virgin females, and none hatched and interpreted this as parthenogenesis. A trial was designed to test for parthenogenesis in *N. huttoni*. Five newly emerged female adults reared from nymphs collected in the field were fed but kept in isolation without a mate. Eggs laid by females were unfertilised and were kept in the laboratory to see whether they hatched. Tests were conducted in February-March 1996 and December 1997.

4.2.8 Seasonal variations in population density

Field data obtained for determining the number of the generations in the field were used to describe seasonal variation in population size.

4.3 Results and discussion

4.3.1 Overwintering of *N. huttoni*

Overwintering stage. At Hornby, *N. huttoni* overwintered as adults of the third (autumn) generation. This is in agreement with the work of Gurr (1957). Although a few 4th or 5th instar nymphs that hatched late and were not able to complete emergence into adults before winter were found in hibernation, they apparently did not survive since only adults were seen at the beginning of spring.

Overwintering is a critical stage for insects. In many cases, high numbers are present at the start of winter, but only a few survive (Elkassabary 1996). Many species of Hemiptera overwinter as adults (Zhang shi-mei 1985) including *Nezara viridula* (Todd 1989), *Plautia affinis* (Coombs & Khan 1998), *Orius insidiosus* (Elkassabary 1996), *Oncopeltus fasciatus* (Dallas) (Sauer & Feir 1973), *Lygaeus equestris* (L.) (Kugelberg 1973), *Anthocoris nemorum*, *A. sarothamni* Douglas and Scott, and *A. confusus* Reuter (Hill 1957, 1960, and 1965). In contrast, Zhang shi-mei (1985) reported that *Cavalerius saccharivorus* overwinters as adults and eggs, *Nysius ericae* as adults and late-instar nymphs, *Pirkimerus japonicus* as adults, eggs and nymphs, *Eurostus validus* as 4th instar nymphs, *Okeanus quelpartensis* and *Pentatoma japonica* as 1st and 2nd instar nymphs, and *Habrochia chinensis* and *Stephanites* sp. as eggs.

A very large proportion of the New Zealand Heteroptera, including Lygaeidae, seems to overwinter as adults and seek specific hibernating shelters (Myers 1926). Eyles (1963d) reported that seven species of Lygaeidae:

Rhyparochrominae, viz. *Scolopostethus affinis*, *S. decoratus*, *S. grandis*, *Stygnocoris fuliginus*, *S. pedestris*, *Drymus sylvaticus*, and *D. brunneus* overwinter as adults, whereas *Scolopostethus thomsoni* overwinters as adults and 3rd to 5th instar nymphs, and *Stygnocoris rusticus* as eggs.

Overwintering time. Adults went into hibernation in early May 1996, mid-April in 1997 and 1998, and late April in 1999. These observations agree with the findings of Gurr (1952) who reported that adults started to overwinter about the end of April in Nelson, 426 kilometres north of Christchurch. With the onset of colder weather, adults retired to the bases of grass-tufts and weeds, and remained there in a more or less inactive state during the winter. In the present study, it was observed that the timing of adult movement into overwintering habitats was affected by rainfall, and was associated with a temperature drop. If the rainy season starts early, as in 1997 and 1998, adults overwinter earlier. When disturbed in cold weather, they were very sluggish, but on sunny, mild days in winter (e.g., at 2:00 pm of 1 July 1996, air temperature 9.5°C, ground temperature 11°C), adults came out of hibernation temporarily and moved slowly. If removed from their hibernation quarters to higher temperature (25°C) in the laboratory, they became very active within a few minutes.

Overwintering habitats and plants. Adults overwintered in sheltered places, such as at the bases of weeds and tufts of grasses, under stones, in cushions of moss, and under fallen leaves. Eyles (1965b) reported that the winter habitat of *N. huttoni* in his study area in Nelson was restricted almost entirely to Yorkshire fog plants. Some *N. huttoni* were found in the crowns of brown top in May, but none were found there in June and July. At my research site, sand spurrey (*Spergularia rubra*), which was one of the main summer host plants, was one of the most important overwintering host plants. *N. huttoni* lived year round under Mexican daisy (*Erigeron karvinskianus* D C) and alyssum (*Lobularia maritima*) at the bases of a house wall, reproducing during

summer and overwintering during winter. In such localities, the bugs were found in large numbers in late autumn (April) and early winter (mainly in May), but the number of adults found decreased as winter progressed (June and July) presumably due to the frequent rainfall.

The overwintering habitats of *N. huttoni* are similar to those of other species of Lygaeidae described by Malipatil (1979) who reported that lygaeids were present and breeding in a field in south-east Queensland, Australia all year round. He found overwintering adults sheltering in the litter, beneath a thick cover of vegetation. The information on overwintering habitats presented here for *N. huttoni* verifies and extends earlier work (Gurr 1957, Eyles 1965b).

Emergence in spring. During the four years of intensive study (1996-1999), overwintered adults were found to emerge from hibernation in late August (1996) or early September (1997, 1998, and 1999). All overwintered adults left their hibernacula in about one week. Gurr (1952) reported that overwintered adults emerged at the beginning of August in the Nelson district. He (Gurr 1965b) observed that on 10 August 1957, adults were numerous and could be found anywhere in the Nelson area. These results indicate that adults leave their overwintering quarters about 3-4 weeks earlier in Nelson than in Christchurch. This may be due to the warmer climate of Nelson. The date of emergence is undoubtedly governed by climatic conditions especially by increasing temperature.

4.3.2 Number of generations per year

Direct field observations. Fig. 4-1 shows the life history of *N. huttoni* in the field observed at 5-day intervals from spring 1995 to autumn 1999. The 4-year results showed that three generations occurred in a year in Canterbury. The developmental duration and periods of occurrence of each life stage in each

Year	Generation	Aug	Sep			Oct			Nov		Dec		Jan		Feb		Mar		Apr		May
1995-1996	Overwintered	+	+	+	+	+	+	+	+												
	1		o	o	o	o	o	o	o												
						~	~	~	~												
	2								+	+	+	+	+	+							
									o	o	o	o	o	o							
											~	~	~	~							
	3													+	+	+	+	+	+		
														o	o	o	o	o	o		
															~	~	~	~	~	~	~
1996-1997	Overwintered		+	+	+	+	+	+	+												
	1			o	o	o	o	o	o												
						~	~	~	~												
	2								+	+	+	+	+	+							
									o	o	o	o	o	o							
											~	~	~	~							
	3												+	+	+	+	+	+			
													o	o	o	o	o	o			
														~	~	~	~	~	~	~	~
1997-1998	Overwintered		+	+	+	+	+	+	+												
	1			o	o	o	o	o	o												
						~	~	~	~												
	2								+	+	+	+	+	+							
									o	o	o	o	o	o							
											~	~	~	~							
	3												+	+	+	+	+	+			
													o	o	o	o	o	o			
														~	~	~	~	~	~	~	~
1998-1999	Overwintered		+	+	+	+	+	+													
	1		o	o	o	o	o	o													
						~	~	~	~												
	2								+	+	+	+	+	+							
									o	o	o	o	o	o							
											~	~	~	~							
	3												+	+	+	+	+	+			
													o	o	o	o	o	o			
														~	~	~	~	~	~	~	~

Fig. 4-1. Life history of *N. huttoni* in the field from 1995 - 1999. Observations (symbols) were made at 10 day intervals. +: adult, o: egg, ~: nymph

generation were also recorded at 5-day intervals from spring 1995 to spring 1999 and the results are summarised in Tables 4-1 and 4-2.

Developmental duration of each generation and life stage in the field. *N. huttoni* completed development of the first generation in about 55-65 days from 10-15 September to 10-15 November (Table 4-1). Eggs laid by overwintered adults on about 10-15 September hatched in 20-25 days. Nymphs completed their development in 30-45 days. Development of the second generation was completed in 35-50 days between 20 November (1995 and 1997) and 15 January (1999). Eggs of the second generation hatched in 10-18 days between 20 November (1995 and 1997) and 23 December (1998), and nymphs completed development in 20-35 days between 30 November (1997) and 15 January (1999). The developmental duration of the third generation was 20-60 days from 10 January (1998) to 20 March (1996). Eggs hatched in 5-15 days between 10 January (1998) and 5 February (1996 and 1999) and the time required for nymphal development was 15-45 days between 15 January (1998) and 20 March (1996).

The time required for development by the first generation (55-65 days) was longer than that required by the second (35-50 days) and third generations (20-60 days). However, the difference in developmental duration between the second and third generations was not large. The time required for development by the second and third generations was about 2/3 of that required by the first generation.

Period of occurrence of each generation and life stage in the field. The period of occurrence for the first generation was about 120-135 days between 10 September (1995, 1996 and 1998) and 25 January (1996 and 1999) (Table 4-2). The periods of occurrence of eggs, nymphs and adults of the first generation were 50-55 days between 10 September (1995, 1996, and 1998) and 5 November (1995, 1996 and 1997), 45-80 days between 1 October

Table 4-1. Developmental duration of *N. huttoni* in the field (in days).

Year	Stage	1st generation		2nd generation		3rd generation	
1995-1996	Egg	10/9/95 - 1/10/95	20	20/11/95 - 5/12/95	15	20/1/96 - 5/2/96	15
	Nymph	1/10/95 - 10/11/95	40	5/12/95 - 10/1/96	35	5/2/96 - 20/3/96	45
	Egg - adult	10/9/95 - 10/11/95	60	20/11/95 - 10/1/96	50	20/1/96 - 20/3/96	60
1996-1997	Egg	10/9/96 - 1/10/96	20	1/12/96 - 15/12/96	15	15/1/97 - 25/1/97	10
	Nymph	1/10/96 - 15/11/96	45	15/12/96 - 5/1/97	20	25/1/97 - 15/2/97	20
	Egg - adult	10/9/96 - 15/11/96	65	1/12/96 - 5/1/97	35	15/1/97 - 15/2/97	30
1997-1998	Egg	15/9/97 - 10/10/97	25	20/11/97 - 30/11/97	10	10/1/98 - 15/1/98	5
	Nymph	10/10/97 - 10/11/97	30	30/11/97 - 25/12/97	25	15/1/98 - 30/1/98	15
	Egg - adult	15/9/97 - 10/11/97	55	20/11/97 - 25/12/97	35	10/1/98 - 30/1/98	20
1998-1999	Egg	10/9/98 - 1/10/98	20	5/12/98 - 23/12/98	18	25/1/99 - 5/2/99	10
	Nymph	1/10/98 - 12/11/98	43	23/12/98 - 15/1/99	23	5/2/99 - 5/3/99	30
	Egg - adult	10/9/98 - 12/11/98	63	5/12/98 - 15/1/99	41	25/1/99 - 5/3/99	40

Table 4-2. Period of occurrence of *N. huttoni* in the field (in days).

Year	Stage	1st generation		2nd generation		3rd generation	
1995-1996	Egg	10/9/95 - 5/11/95	55	20/11/95 - 10/1/96	50	20/1/96 - 20/3/96	60
	Nymph	1/10/95 - 15/12/95	75	5/12/95 - 25/1/96	50	5/2/96 - 20/5/96	105
	Adult	10/11/95 - 10/1/96	60	10/1/96 - 20/3/96	70	20/3/96 - 15/5/96 15/5/96 - 30/8/96 1/9/96 - 5/11/96 20/3/96 - 5/11/96	Before winter: 55 Over winter: 105 After winter: 65 Total: 225
	Egg - adult	10/9/95 - 10/1/96	120	20/11/95 - 20/3/96	120	20/1/96 - 5/11/96	290
1996-1997	Egg	10/9/96 - 5/11/96	55	1/12/96 - 25/1/97	55	15/1/97 - 5/3/97	50
	Nymph	1/10/96 - 20/12/96	80	15/12/96 - 20/2/97	65	25/1/97 - 5/4/97	70
	Adult	15/11/96 - 25/1/97	70	5/1/97 - 5/3/97	60	15/2/97 - 10/4/97 10/4/97 - 30/8/97 1/9/97 - 5/11/97 15/2/97 - 5/11/97	Before winter: 55 Over winter: 143 After winter: 65 Total: 263
	Egg - adult	10/9/96 - 25/1/96	135	1/12/96 - 5/3/96	95	15/1/97 - 5/11/97	295
1997-1998	Egg	15/9/97 - 5/11/97	50	20/11/97 - 15/1/98	55	10/1/98 - 10/2/98	30
	Nymph	10/10/97 - 25/11/97	45	1/12/97 - 20/1/98	50	15/1/98 - 1/3/98	45
	Adult	10/11/97 - 15/1/98	65	25/12/97 - 10/2/98	45	1/2/98 - 15/4/98 15/4/98 - 30/8/98 1/9/98 - 30/10/98 1/2/98 - 30/10/98	Before winter: 75 Over winter: 135 After winter: 60 Total: 270
	Egg - adult	15/9/97 - 15/1/98	120	20/11/97 - 10/2/98	80	10/1/98 - 30/10/98	290
1998-1999	Egg	10/9/98 - 30/10/98	50	25/11/98 - 25/1/99	60	25/1/99 - 25/3/99	60
	Nymph	1/10/98 - 10/12/98	70	23/12/98 - 23/2/99	62	15/2/99 - 25/4/99	70
	Adult	15/11/98 - 25/1/99	70	15/1/99 - 25/3/99	70	5/3/99 - 30/4/99 30/4/99 - 30/8/99 1/9/99 - 5/11/99 5/3/99 - 5/11/99	Before winter: 56 Over winter: 123 After winter: 66 Total: 245
	Egg - adult	10/9/98 - 25/1/99	135	25/11/98 - 25/3/99	120	25/1/99 - 5/11/99	285

(1995,1996 and 1998) and 20 December (1996), and 60-70 days between 10 November (1995 and 1997) and 25 January (1997 and 1999), respectively.

The second generation with a period of occurrence of 80-120 days occurred between 20 November (1995 and 1997) and 25 March (1999). Eggs of the second generation occurred over a period of 50-60 days between 20 November (1995 and 1997) and 25 January (1997 and 1999), nymphs, 50-65

days between 1 December (1997) and 20 February (1999), and adults, 45-70 days between 25 December (1997) and 25 March (1999).

Third generation, eggs had a period of occurrence of 30-60 days and were found between 10 January (1998) and 25 March (1999), while nymphs occurred over 45-105 days between 15 January (1998) and 20 May (1996). Because *N. huttoni* overwinters as adults, the period of occurrence for the adults of the third generation was the longest of the three generations.

The occurrence period of adults of the third generation consisted of three stages: before winter, over winter, and after winter. Adults were found before winter (from emergence to overwintering) over about 55-75 days between the beginning of February (1998) and mid-May (1996); the overwintering period was about 105-143 days from 10 April (1997) to 30 August (1996-1999); and after winter, the overwintered adults emerged from their overwintering sites at the beginning of September. They were found until the beginning of November over a period of 60-65 days. The occurrence period for adults before winter was about 10 days shorter than that after winter. In total, adults of the third generation were present for about 225-270 days between early February (1998) and early November (1996-1999). The total occurrence period of the third generation from egg to adult was 285-295 days between 10 January (1998) and early November (1996-1999). However, the occurrence period of egg to adult of the third generation before winter (85-115 days), which occurred between about 10 January (1998) and mid-May (1996), was very similar to that of the second generation (80-120 days) in length.

Field sampling. Twenty-eight samples were taken in 1995-1996 and 22 in 1996-1997. Results are shown in Fig. 4-2. Three peaks of nymphal numbers are indicated by n_1 , n_2 , and n_3 and those for adults by a_1 , a_2 , and a_3 during the 1995-1996 and 1996-1997 seasons. Each peak represents one generation.

These results coincide with those obtained from the direct field observations, as noted above.

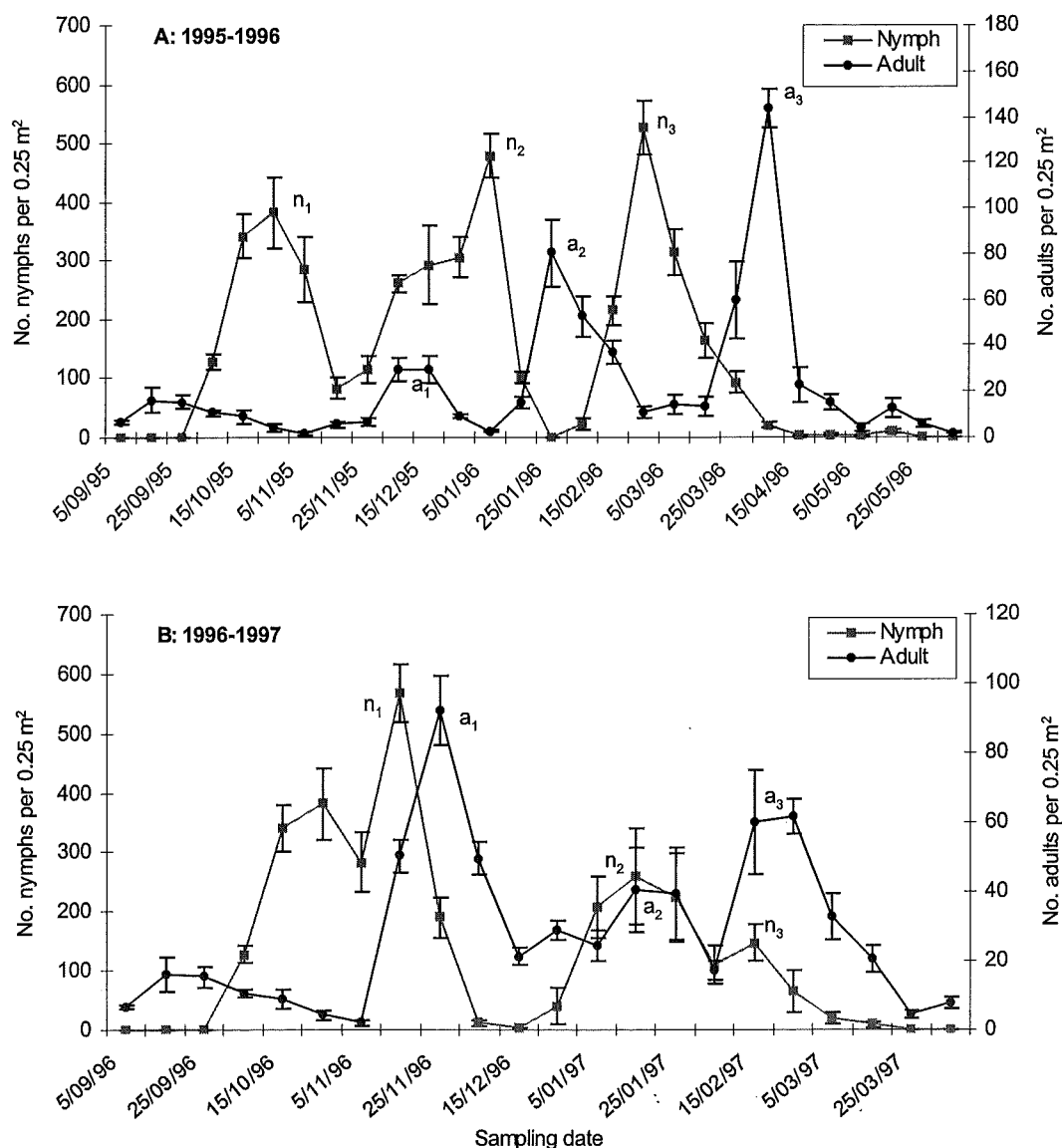


Fig. 4-2. Seasonal abundance of *N. huttoni* adults and nymphs (mean \pm S.E.) in the field in 1995-1996 (A) and 1996-1997 (B). Samples were taken at 10-day intervals starting on 5 September in both seasons. Twenty eight samples were obtained in 1995-1996 and 22 samples in 1996-1997. n_1 , n_2 , and n_3 indicated three peaks of occurrence of nymphs in numbers throughout the seasons; and a_1 , a_2 , and a_3 three peaks of adults.

It can also be seen that each nymphal peak was followed by an adult peak, which was very apparent in 1995-1996. In 1995-1996 (Fig. 4-2 A), three peaks of occurrence for nymphs (n_1 , n_2 , and n_3) appeared on 25 October, 5 January and 25 February, respectively, followed by three peaks of occurrence for adults (a_1 , a_2 , and a_3) on 5-15 December, 25 January and 5 April, respectively. In 1996-1997 (Fig. 4-2 B), three peaks of occurrence for nymphs (n_1 , n_2 , and n_3) were recorded on 15 November, 15 January and 15 February, respectively, followed by three peaks for adults on 25 November, 15-25 January and 25 February. These findings are consistent with the results obtained from field observations shown in Fig. 4-1 and Table 4-2.

The four years of field observations (Fig. 4-1) and two years of sampling (Figs. 4-3 and 4-4) indicate that *N. huttoni* shows some generation overlap. Nymphs of the first and second generations overlapped for about 10 days between 5 and 15 December 1995 (Figs. 4-1 and 4-3 A), and about 5 days between 15 and 20 December 1996 (Figs. 4-1 and 4-4 A). However, no overlap occurred in 1997-1998 and 1998-1999 (Fig. 4-1). Occurrence of the adults of the first and the second generation had an overlap period of 20 days between 5 and 25 January 1997 (Figs. 4-1 and 4-4 B), 20 days between 25 December 1997 and 15 January 1998, and 10 days between 15 and 25 January 1999. No overlap of adults occurred in 1995-1996 (Fig. 4-1).

Overlap also occurred between the second and third generations. The overlap period of their nymphs was about 25 days between 25 January and 20 February 1997 (Figs. 4-1 and 4-4 A), 5 days between 15 and 20 January in 1998, and 10 days between 15 and 25 February 1999 (Fig. 4-1). No overlap occurred in 1996. Adults of the second and the third generation had an overlap of 20 days in 1996-1997 and 1998-1999, between 15 February and 5 March 1997 and between 5 and 25 March 1999, respectively. In 1997-1998, the overlap period of adults belonging to these two generations was about 10 days

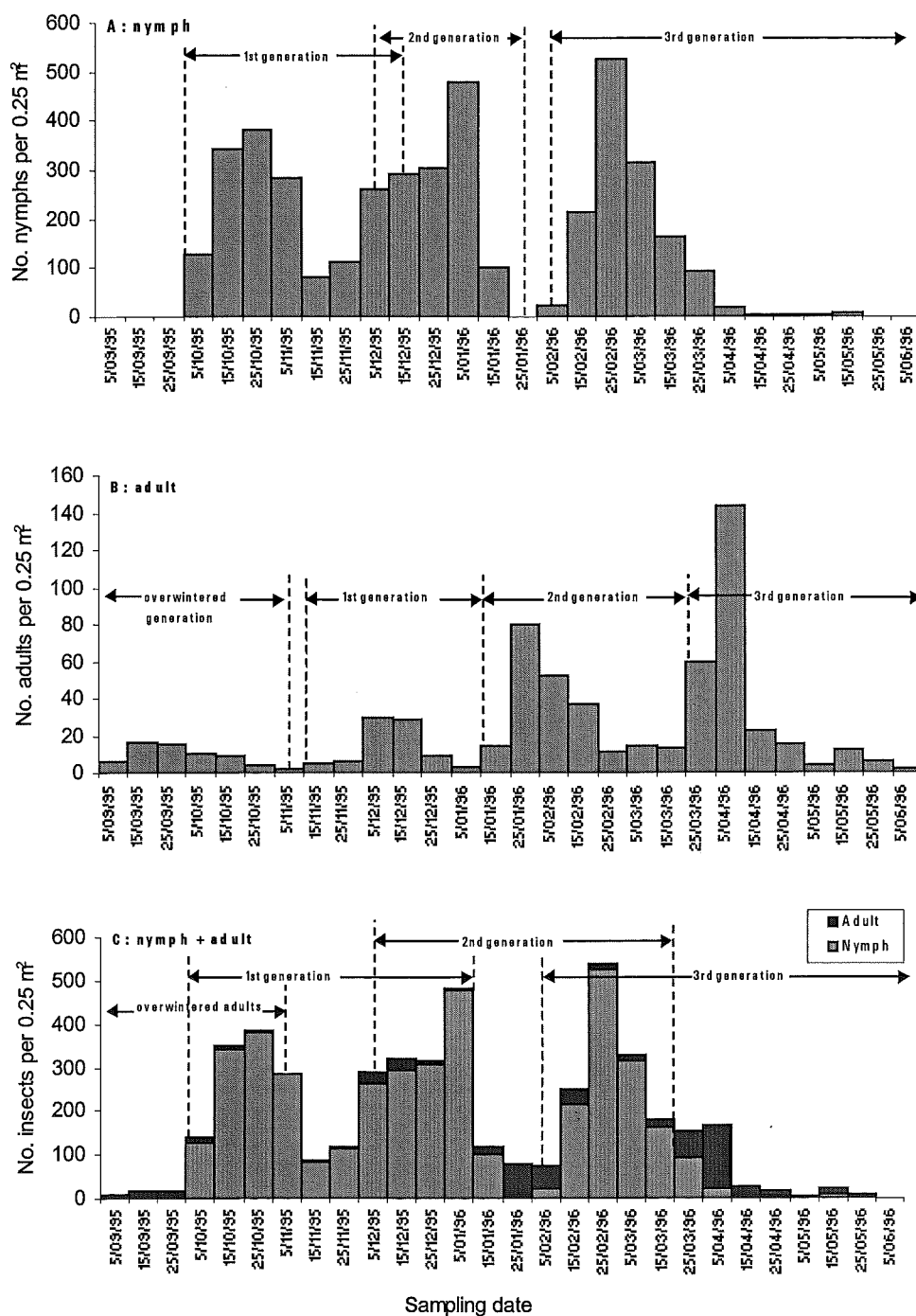


Fig. 4-3. Seasonal occurrence of nymphs and adults of *N. huttoni* in the field in 1995-1996.

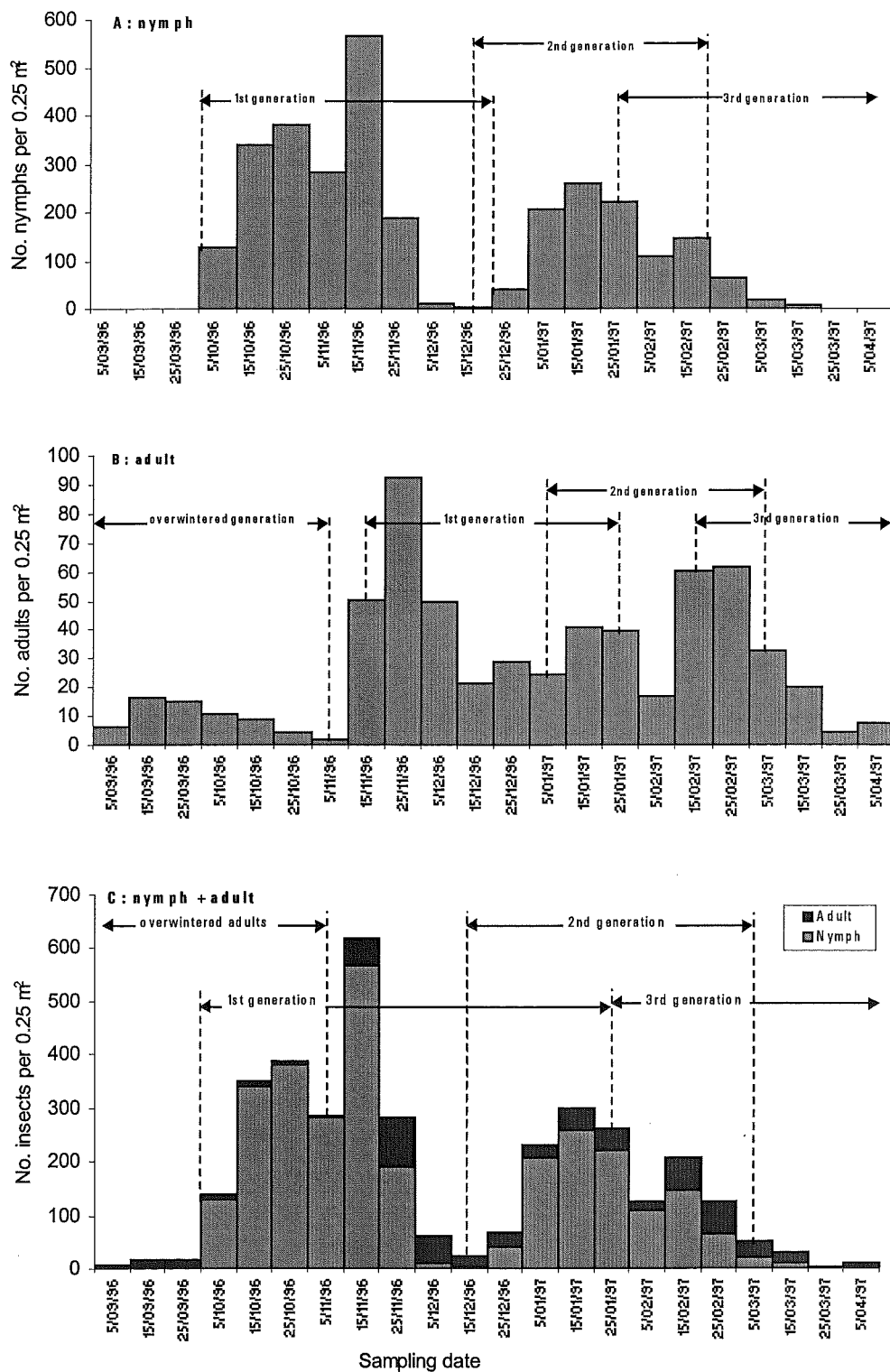


Fig. 4-4. Seasonal occurrence of nymphs and adults of *N. huttoni* in the field in 1996-1997.

between 1 and 10 February 1998 (Fig. 4-1). However, no overlap period was found between overwintered and first-generation adults.

There may be two reasons for the occurrence of generation overlaps in *N. huttoni*. Firstly, the adults live for some time and have a long period of oviposition. During the four years of field observations, overwintering adults in spring, and first- and second-generation adults in summer were present for ca. two months (Fig. 4-1 and Table 4-2). Thus, there were large differences in ages of individuals within the same generation, and this could be expected to result in an overlap between generations. Secondly, high summer temperatures accelerated the development of the second and third generations, resulting in that part of the second-generation population that completed development earlier, overlapping with the first-generation population. Similarly, parts of the second and third generations overlapped.

Because of the generation overlaps, interpretation of the life history of *N. huttoni* is difficult and the divisions between generations, especially between the second and third generations, become easily indistinguishable. It would be easy to conclude that two generations occur in this species.

As indicated in Chapter 1, different authors have stated that different number of generations occur in *N. huttoni*. That one generation can occur per year was indicated by Myers (1926) and two generations were stated by Gurr (1952). However, neither gave the experimental evidence supporting these suggestions. Eyles (1963b) reared *N. huttoni* in a greenhouse for a complete breeding season in Palmerston North, North Island, and recorded four generations. Although the greenhouse was not artificially heated, the rearing temperature was higher than in the field, so development of the insects would have been faster. Based on field observations, Eyles (1965b) reported that, *N. huttoni* completes at least three generations a year in Nelson, and he deduced that there was theoretically time for four generations in the field. Farrell &

Stufkens (1993), who conducted their field experiments at Lincoln near Christchurch, about 15 kilometres from my research site in Hornby, reported two generations using a method of sampling insects similar to one of the methods I used in my research (see 'field sampling' in section (2) of "materials and methods" in this Chapter).

In my research, the number of generations was determined in two ways: by direct field observations, which were conducted for four years during 1995-1996 and 1998-1999, and by field sampling during 1995-1996 and 1996-1997. I concluded that in all years three generations occurred.

As shown earlier, second and third generations developed faster than the first generation. Differences in development time between generations may be influenced by physical factors, especially temperature, which affects development rates as the season progresses. The first generation completed development in spring (mid-September to mid-November) during which the mean daily temperature at Christchurch airport was 9.3°C in September, 11.8°C in October, and 12.9°C in November. In contrast, development of the second and third generations was completed between early summer (December) and late summer (February) or the beginning of autumn (March). The mean daily temperatures during this period were 15.7°C in December, 17.0°C in January, 17.6°C in February, and 15.3°C in March. Higher summer temperatures can be expected to result in faster development of the second and third generations.

Here, it is necessary to point out that, to various degrees, all environmental conditions, whether biotic or abiotic elements, terrestrial or aquatic, change over time. These changes can be long or short term, cyclic or acyclic, mild or severe, as well as widespread or localized. The number of generations completed by an insect species a year is greatly influenced by these changes. These changes can affect the length of the life cycle by altering the growth rate

of the insects. A favourable environment can shorten the time of development from egg to adult. Under unfavourable environmental conditions, generation time may become longer.

Among these biotic and abiotic factors, temperature and food (their availability and quality) are two important factors (Yazdani & Agarwal 1997). As with other insects, the number of generations per year is dependent on degree-day accumulations. These two factors affect the developmental rates of individuals and their overall populations. Especially under conditions with sufficient food, environmental temperature serves as the primary stimulus regulating the seasonal cycles of insects. Food supply not only affects mean relative growth rates of insects (Neumann 1986), but many insects have also evolved different ways of using seasonal variability in food quantity and quality to co-ordinate life cycle events (Tauber *et al.* 1986). There are also other factors that have important influences on insect seasonal cycles such as photoperiod, humidity, and population density. Thus, the number of generations of the same species may vary with the year, host plants, and locality. For example, *Anthocoris sarothamni* Douglas and Scott (Hemiptera: Anthocoridae) produces one generation under normal summer conditions, but two generations may occur during exceptionally fine summers (Hill 1961). For *N. huttoni*, in the seasons in which the average temperatures falls below that under which these studies were conducted, the number of generations may be less, or with higher average temperatures, may become greater.

4.3.3 Seasonal copulation activities in the field

Assessment of copulation numbers of *N. huttoni* adults in the field during 1995-1996 and 1998-1999 is shown in Fig. 4-5. Copulation of the overwintered adults started at the beginning of September and ended later October; peaks of copulation appeared around 20 September each year. Little difference in

Year	Generation	Sep			Oct			Nov		Dec		Jan		Feb		Mar		Apr		May
1995-1996	Overwintered	1	2	3	2	1	1	0												
	1								3	2	2	3	1	1						
	2											2	3	2	2	2	1	0		
	3																1	0	0	0
1996-1997	Overwintered	1	2	3	2	1	1	0												
	1								1	2	3	2	2	2						
	2											1	2	3	1	1	1			
	3													0	1	1	0	0	0	
1997-1998	Overwintered	1	2	3	2	1	1													
	1								1	3	3	2	2	1	0					
	2										0	3	2	2						
	3													1	1	1	1	1	0	0
1998-1999	Overwintered	1	2	3	2	1	0													
	1								1	2	3	2	2	2	1					
	2											0	1	2	2	2	1	0	0	
	3															0	0	0	0	0

Fig. 4-5. Copulation activity of *N. huttoni* adults in the field from 1995-1999. Observations were made at 10 day intervals. **0**: no copulation occurred; **1**: a few adults copulated; **2**: many adults copulated; **3**: very many adults copulated (peak of copulation).

the time of emergence of adults in spring was found between years and no difference was found in the time of copulation activities, especially the appearance of the copulation peak each year. Copulation activities of adults of the first generation occurred between mid-November and early or mid-January and peaks of copulation were between mid-November (1995 and 1997) and early December (1996 and 1998). In 1995-1996, adults of the first generation had two copulation peaks around mid-November and mid-December 1995. For the second generation, the adults commenced copulation in early January (1997 and 1998) or mid-January (1996) or later January (1999), and ended around later February (1997) or the beginning of March (1996 and 1999). In 1997-1998, the period of copulation for adults of the second generation was very short (about one month) and occurred in January 1998. Peaks of copulation for adults of the second generation appeared from early (1997-1998) to late (1995-1996 and 1996-1997) January. For the third generation, no adults (1998-1999) or very few of them (1995-1996 and 1997-1998), were found copulating.

It is noteworthy that adults of the third generation (overwintered adults) did not copulate before overwintering, the rare exception being those few adults that emerged early (1997 and 1998) (Fig. 4-5). Copulation normally occurs in early spring and as will be discussed later, is probably associated with reproductive diapause of the adults. Since field observations showed that copulation in *N. huttoni* occurred at high temperatures, the lower temperatures in autumn were probably not favourable for copulation.

N. huttoni had a distinct seasonal pattern in copulation (Fig. 4-5). Copulating adults were found throughout spring and summer, with three distinct mating periods occurring between early September and late February or early March. For about 10 days between about 25 October and 10 November, no copulation was seen in the field in all four years, as adults were absent. This

period was when overwintered adults had disappeared and first-generation adults had yet to appear.

4.3.4 Seasonal oviposition activities in the field

A search for oviposition sites has been made in soil and on different host plants. The eggs have been found lying in soil, but none in or on plants.

Results of dissections of female adults for determining the number of individuals with mature eggs on various sampling dates in 1997-1998 and 1998-1999 are summarised in Table 4-3. In early November of both years, presence of overwintered adults ended before first-generation adults emerged, and no adults were found in the field on 5 November. Seasonal variation of oviposition in the field is shown in Fig. 4-6.

In 1997-1998 (Fig. 4-6 A), oviposition by overwintered adults commenced in early September. The number of ovipositing adults increased as spring progressed and reached a peak in late September. The oviposition peak, during which all 30 dissected adults were found to have mature eggs, lasted for about one month from late September to late October. Oviposition activities of overwintered adults ended when they disappeared from the field around the end of October.

First-generation adults, which initially emerged around 10 November 1997 (Fig. 4-1) started to lay eggs in mid-November and reached the oviposition peak in numbers in early December (also see Table 4-3). The peak lasted for about 10 days. Because second-generation adults that emerged in late December (1997) were added to the population of first-generation adults (Fig. 4-1), the adult population in the field from late December 1997 to mid-January 1998 was a mixture of first-generation adults in a stage of oviposition peak and

Table 4-3. Results of dissection of female adults of *N. huttoni* in 1997-1998 and 1998-1999.

1997 - 1998				1998 - 1999			
Sampling date	Generation	No.adults dissected	No.adults with eggs	Sampling date	Generation	No.adults dissected	No.adults with eggs
5/09/97	overwintered	30	13	5/09/98	overwintered	30	15
15/09/97	overwintered	30	22	15/09/98	overwintered	30	28
25/09/97	overwintered	30	30	25/09/98	overwintered	30	30
5/10/97	overwintered	30	30	5/10/98	overwintered	30	30
15/10/97	overwintered	30	30	15/10/98	overwintered	30	29
25/10/97	overwintered	30	29	25/10/98	overwintered	30	28
5/11/97*	---	---	---	5/11/98*	---	---	---
15/11/97	1	30	5	15/11/98	1	30	0
25/11/97	1	30	24	25/11/98	1	30	5
5/12/97	1	30	30	5/12/98	1	30	17
15/12/97	1	30	30	15/12/98	1	30	28
25/12/97	1 & 2	30	19	25/12/98	1	30	30
5/01/98	1 & 2	30	11	5/01/99	1	30	30
15/01/98	2	30	19	15/01/99	2 & 1	30	5
25/01/98	2	30	9	25/01/99	2 & 1	30	8
5/02/98	2 & 3	30	7	5/02/99	2	30	2
15/02/98	3	30	2	15/02/99	2	30	2
25/02/98	3	30	9	25/02/99	2	30	4
5/03/98	3	30	9	5/03/99	2 & 3	30	3
15/03/98	3	30	5	15/03/99	2 & 3	30	2
25/03/98	3	30	2	25/03/99	2 & 3	30	2
5/04/98	3	30	0	5/04/99	3	30	0
15/04/98	3	30	0	15/04/99	3	30	0

* No adults occurred in the field on 5 November 1997 and 1998.

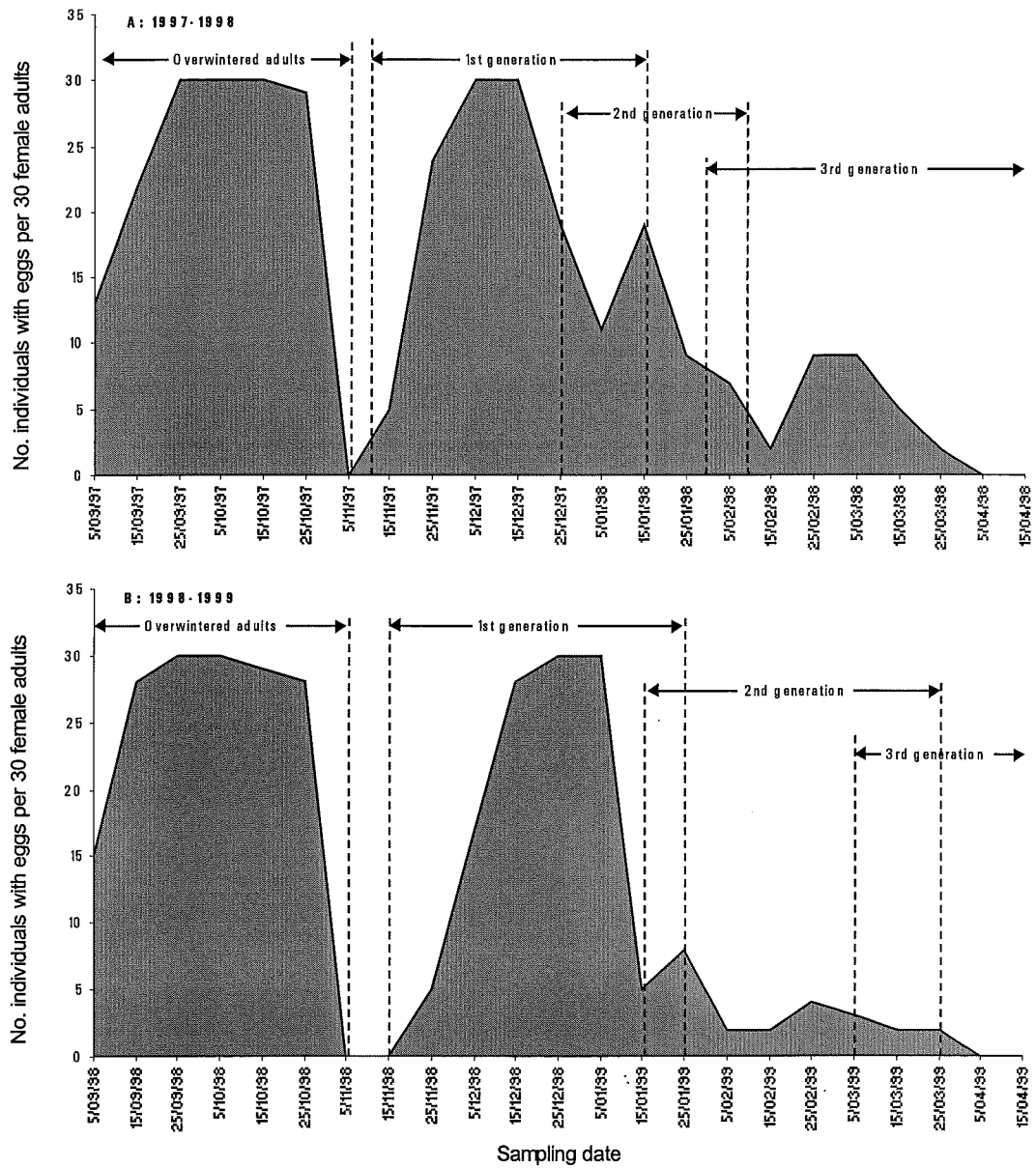


Fig. 4-6. Numbers of female *N. huttoni* with eggs in the field in 1997-1998 and 1998-1999.

second-generation adults with differing maturity and reproductive status. Adults of the first and second generation had an overlap period of about 20 days from 25 December 1997 to 15 January 1998. Ovipositing adults started to decline in numbers from late December 1997 as the second-generation adults emerged (Table 4-3). The number of ovipositing adults started to increase again from early January 1998 as the second-generation adults became mature and reached a peak in mid-January when 19 of the 30 adults dissected could have oviposited. This peak lasted for about one week and was followed by a decline in numbers of ovipositing adults.

The proportion of ovipositing adults in the third generation was much lower, with a maximum of 9 individuals with mature eggs per 30 dissected adults from late February to early March 1998. Those adults were possibly long-lived members of the second generation. No third-generation adults were found to have mature eggs after the beginning of April 1998. Therefore, the third-generation females exhibited no reproductive maturity or activity before the onset of winter.

The numbers of females that oviposited in 1998-1999 (Fig. 4-6 B) showed a very similar seasonal pattern to that recorded in 1997-1998. Thus, 50% of the overwintered adults were found to have mature eggs in early September 1998. The oviposition peak appeared about mid-September and lasted for about 40 days. The reproductive activity of overwintered adults ended by the end of October. First-generation adults started to lay eggs on around 20 November 1998. The oviposition peak, which occurred 10 days later and lasted 10 days longer than in 1997-1998, appeared between mid-December 1998 and early January 1999. The period of overlap of adults in the first and second generations (15-25 January 1999) was 10 days shorter than in 1997-1998 (Fig. 4-1). On 25 January 1999, a maximum of 8 individuals out of 30 dissected females collected from a population, consisting of first- and second-

generation adults, were found to have mature eggs. This might be regarded as the oviposition peak of the second-generation adults.

The period of peak oviposition of overwintered adults (early and mid-spring) was followed by a rapid decline in their numbers, and adults were absent for only 5-10 days before first-generation adults appeared (Figs. 4-3 B, 4-4 B, and 4-6). First-generation adults did not contribute to the overwintering population.

First-generation adults started to emerge in early to mid-November and had a period of occurrence of 60-70 days. Time of emergence differed between different years by about one week. Second-generation adults began to emerge between late December and mid-January and had a period of occurrence of 45-70 days. A difference of about three weeks was found in the time of emergence between years. Third-generation adults, which emerged between early February and mid-March, had the greatest difference - one and a half months in time of emergence between years. Therefore, a proportion of the second-generation adults contributed to the first-generation population, whereas, a proportion of the third-generation adults, which emerged early, contributed to the second-generation population. This made second-generation adults have periods of overlap with first-generation adults early on and third-generation adults later. This may be the reason why the oviposition peak of second-generation adults was less apparent than the peaks for overwintered and first-generation adults.

Fig. 4-6 shows there were three oviposition peaks each year. The first one consisting of overwintered adults had the longest duration of about 30-40 days. The second one consisting of first-generation adults lasted for about 10-20 days, whereas the third one appeared during the occurrence period of second-generation adults and lasted for only about 5 days. Oviposition activities of *N. huttoni* were therefore maximal in the early part of the season (spring and early summer). Third-generation adults oviposited in spring after

hibernation. This activity is associated with the onset of reproductive diapause, which is discussed in Chapter 8.

Oviposition activity of *N. huttoni* exhibited seasonal variation similar to that of sexual activity, as indicated by the field-observations (Fig. 4-5). Thus, mating activity was closely associated with oviposition activity. This demonstrates an inseparability of mating and oviposition in this species.

4.3.5 Sex ratio

Sex ratio in field-collected and laboratory-emerged population. Eight samples of late-instar nymphs were taken from the field between 14 November 1997 and 11 February 1998. No deaths occurred before ecdysis to the adult. A total of 587 adults (287♀ + 300♂) emerged in the laboratory. Two of these samples containing 193 adults (93♀ + 100♂) were from the first-generation population, while four with 246 adults (124♀ + 122♂), and two with 148 adults (70♀ + 78♂) belonged to the second- and third-generation populations, respectively.

Sex ratios of the 8 samples ranged from 1:0.86 to 1:1.22, and the total sex ratio of 587 adults (287♀ : 300♂) was 1:1.05 (Table 4-4). No significant differences from a 1:1, ♀:♂ ratio were found in the 8 samples or the total adult population ($P > 0.05$, χ^2 test). Because the adults used to determine the sex ratio had emerged from late-instar nymphs collected from the field, it is reasonable to deduce that the intrinsic sex ratio of the species is 1:1.

In Hemiptera, species whose sex ratios (♀:♂) have been recorded to be very close to that of *N. huttoni* are *Oncopeltus fasciatus* (Dallas): 1:1 (Sauer & Feir 1973), *Nysius ericae*: 1:1.13-1:1.18, *Cyclopelta obscura*: 1:1, and *Eurydema gebleri* Kolenati: 1:1 (Zhang shi-mei 1985).

Table 4-4. Sex ratio of *N. huttoni* adults that emerged from late-instar nymphs collected from the field in 1997-1998.

Sampling date	Generation	No. adults	No. females	No. males	F : M
14/11/97	1	106	52	54	1 : 1.04
19/11/97	1	87	41	46	1 : 1.12
24/12/97	2	46	22	24	1 : 1.09
9/01/98	2	36	18	18	1 : 1.00
10/01/98	2	104	56	48	1 : 0.86
15/01/98	2	60	28	32	1 : 1.14
5/02/98	3	128	61	67	1 : 1.10
11/02/98	3	20	9	11	1 : 1.22
Total	-----	587	287	300	1 : 1.05

Proportions of females and males measured were compared with the expected 1:1 (F:M) ratio using a chi-squared test. None was significantly different ($P > 0.05$).

Sex ratio in the laboratory-reared population. *Sex ratio in samples reared at different temperatures.* Table 4-5 shows the sex ratios of adults produced at 6 temperatures in the laboratory. In all cases, the sex ratio did not differ significantly from 1:1 ($P > 0.05$, χ^2 test). Lab results were therefore in agreement with the sex ratio of bugs collected from the field which emerged in the laboratory (Table 4-4).

Sex ratio of populations produced under different photoperiods. Table 4-6 shows the sex ratios of adults produced under three different photoperiods at 20 and 27.5°C. The data indicate that photoperiod seems to affect the sex ratio of *N. huttoni*. At 20°C, the proportion of adult males increased as the photophase increased, and the 8-h photoperiod produced a male-biased sex ratio of 1:1.86 ($P < 0.05$, χ^2 test). At 27.5°C, the 12-h photoperiod produced a male-biased sex ratio of 1:1.73 ($P < 0.01$). The sex ratios of the population produced at 16-h photoperiod were 1:0.69 at 20°C and 1:1.08 at 27.5°C, and the number of males was not significantly different from that of females ($P >$

0.05). The results indicate that a short-day photoperiod combined with low temperature produced the greatest proportion of males.

Table 4-5. Sex ratio of *N. huttoni* reared at different temperatures.

Temperature (°C)	No. adults	No. females	No. males	F : M
15	19	10	9	1 : 0.90
20	81	35	46	1 : 1.31
25	47	22	25	1 : 1.14
30	111	57	54	1 : 0.95
35	212	103	109	1 : 1.06
L - T	120	63	57	1 : 0.90
Total	590	290	300	1 : 1.03

Proportions of females and males measured were compared with the expected 1:1 (F:M) ratio using a chi-squared test. None was significantly different ($P > 0.05$).

Table 4-6. Sex ratio of *N. huttoni* reared under different photoperiods.

Temperature (°C)	Photoperiod (L:D)	No. adults	No. females	No. males	F : M
20	16 : 8	71	42	29	1 : 0.69
	12 : 12	81	35	46	1 : 1.31
	8 : 16	40	14	26	1 : 1.86*
	Total	192	91	101	1 : 1.11
27.5	16 : 8	104	50	54	1 : 1.08
	12 : 12	112	41	71	1 : 1.73**
	8 : 16	92	46	46	1 : 1.00
	Total	308	137	171	1 : 1.25*

Proportions of females and males measured were compared with the expected 1:1 (F:M) ratio using a chi-squared test. * $P < 0.05$, ** $P < 0.01$.

Hoelscher & Vinson (1971) studied the effect of temperature and photoperiod on the sex ratio of *Campoletis perdistinctus* (Hymenoptera: Ichneumonidae). They found that temperature did not influence the sex ratio of this species, but that photoperiod did. In their study, a 12-h photoperiod produced offspring with a greater percentage of females than any other combinations tested. Fleischer & Gaylor (1988) reported that the total number of females ($n = 218$) and males ($n = 210$) of *Lygus lineolaris* reared at seven constant temperatures of 18, 19, 20, 22, 25.5, 26.5, and 30°C in the laboratory conformed to a sex ratio of 1:1. Deviations from 1:1, however, have been observed in the field (Ridgeway & Gyrisco 1960).

Sex ratio in field-collected population. In the 1995-1996 season, the first sample was taken on 5 December. Sampling of overwintered and partial first-generation adults therefore was missed. In 1996-1997 and 1997-1998, 18 and 22 samples were obtained, respectively, starting on an earlier date. The results are presented in Table 4-7.

The proportion of males to females (1:1.28-1.95) in 5 of 17 samples in 1995-1996 was significantly greater than 1:1 ($P < 0.05$ and $P < 0.01$, χ^2 test). These samples were taken on 15 January, 4 February, 15 and 25 March, and 5 April 1996, respectively. The proportion of males in the other 12 samples was not significantly different from that of females ($P > 0.05$). The total sex ratio of 17 samples for that year, which comprised a total of 3213 adults (1438♀ + 1775♂), was 1:1.23 and the departure from 1:1 ratio was statistically significant in favour of males ($P < 0.01$). Similarly, in 1996-1997, the sex ratio of 18 combined samples containing a total of 4823 adults (2295♀ + 2528♂) was 1:1.10 with an excess of males over females ($P < 0.01$). In four of these samples, males were more abundant than females (♀:♂ = 1:1.33-1.56, $P < 0.05$ and $P < 0.01$). In 1997-1998, 4616 adults (2257♀ + 2359♂) from 22 samples were examined. The excess of males in the field was significant (♀:♂

Table 4-7. Sex ratio of *N. huttoni* collected directly from the field.

Sampling date	Generation	No. adults	No. females	No. males	F : M
1995-1996					
5/12/95	1	119	62	57	1 : 0.92
15/12/95	1	222	103	119	1 : 1.11
24/12/95	1	58	28	30	1 : 1.07
15/01/96	2	121	41	80	1 : 1.95**
24/01/96	2	393	204	189	1 : 0.93
4/02/96	2	666	292	374	1 : 1.28**
14/02/96	2	182	81	101	1 : 1.25
24/02/96	2	55	30	25	1 : 0.83
6/03/96	2	70	34	36	1 : 1.06
15/03/96	2 & 3	66	24	42	1 : 1.75*
25/03/96	3	660	269	391	1 : 1.45**
5/04/96	3	86	30	56	1 : 1.87**
17/04/96	3	196	85	111	1 : 1.31
25/04/96	3	63	30	33	1 : 1.10
6/05/96	3	129	61	68	1 : 1.11
15/05/96	3	99	51	48	1 : 0.94
24/05/96	3	28	13	15	1 : 1.15
Total	-----	3213	1438	1775	1 : 1.23**
1996-1997					
5/09/96	overwintered	147	72	75	1 : 1.04
15/09/96	overwintered	441	230	211	1 : 0.92
25/09/96	overwintered	353	138	215	1 : 1.56**
5/10/96	overwintered	375	186	189	1 : 1.02
15/11/96	1	476	218	258	1 : 1.18
25/11/96	1	437	215	222	1 : 1.03
5/12/96	1	245	134	111	1 : 0.83
16/12/96	1	244	125	119	1 : 0.95
6/01/97	1 & 2	265	133	132	1 : 0.99
15/01/97	1 & 2	240	106	134	1 : 1.26
25/01/97	2	200	86	114	1 : 1.33*
7/02/97	2	200	106	94	1 : 0.89
15/02/97	2	200	94	106	1 : 1.13
25/02/97	2 & 3	200	94	106	1 : 1.13
10/03/97	3	200	85	115	1 : 1.35*
15/03/97	3	200	106	94	1 : 0.89
25/03/97	3	200	79	121	1 : 1.53**
5/04/97	3	200	88	112	1 : 1.27
Total	-----	4823	2295	2528	1 : 1.10**

Table 4-7. Continued.

Sampling date	Generation	No. adults	No. females	No. males	F : M
1997-1998					
5/09/97	overwintered	152	86	66	1 : 0.77
15/09/97	overwintered	298	153	145	1 : 0.95
25/09/97	overwintered	200	82	118	1 : 1.44*
5/10/97	overwintered	65	31	34	1 : 1.10
15/10/97	overwintered	116	54	62	1 : 1.15
25/10/97	overwintered	125	51	74	1 : 1.45*
25/11/97	1	195	101	94	1 : 0.93
16/11/97	1	208	118	90	1 : 0.76
5/12/97	1	663	356	307	1 : 0.86
15/12/97	1	200	98	102	1 : 1.04
25/12/97	1 & 2	142	67	75	1 : 1.12
5/01/98	1 & 2	200	110	90	1 : 0.82
15/01/98	1 & 2	200	113	87	1 : 0.77
25/01/98	2	200	100	100	1 : 1.00
5/02/98	2 & 3	200	95	105	1 : 1.11
15/02/98	3	308	137	171	1 : 1.25
25/02/98	3	422	176	246	1 : 1.40*
5/03/98	3	200	95	105	1 : 1.11
15/03/98	3	100	46	54	1 : 1.17
25/03/98	3	100	42	58	1 : 1.38
5/04/98	3	200	91	109	1 : 1.20
35900	3	122	55	67	1 : 1.22
Total	-----	4616	2257	2359	1 : 1.05

Proportions of females and males measured were compared with the expected 1:1 (F:M) ratio using a chi-squared test. * $P < 0.05$, ** $P < 0.01$.

= 1: 1.40-1.45, $P < 0.05$ and $P < 0.01$) in 3 samples. The overall sex ratio in these samples was 1:1.05, and no significant difference was found ($P > 0.05$).

A comparison of annual sex ratios in the three years shows there were variations from year to year. In 1995-1996 and 1996-1997, overall annual sex ratios were 1: 1.23 and 1:1.10 respectively, both being male-biased (Table 4-7, $P < 0.01$, χ^2 test), whereas total sex ratio for the year 1997-1998 was 1:1.05, which was not significantly male-biased ($P > 0.05$).

The sex ratios in four samples collected in spring between 5 September and 5 October 1996 and six samples collected between 5 September and 25 October 1997 reflected the proportions of females to males of the overwintered adults (Table 4-7). Although the sex ratios in one of four samples in 1996-1997 and two of six samples in 1997-1998 were significantly male-biased ($P < 0.05$ and 0.01 , χ^2 test), the total sex ratios for these 4 samples (1996-1997), which comprised a total of 1316 adults ($626\text{♀} + 690\text{♂}$), and the 6 samples (1997-1998) which comprised a total of 956 adults ($457\text{♀} + 499\text{♂}$) were 1:1.0 and 1:1.09, respectively ($P > 0.05$). These results indicate there was no large difference in mortality of females and males during winter, suggesting they are similarly tolerant to cold.

Eyles (1963b) reported that over a complete breeding season (October to April), the total ratio of males to females of *N. huttoni* reared was 50:49. In November, there were more males ($\text{♀}:\text{♂} = 6:12$), whilst in February, there were more females ($\text{♀}:\text{♂} = 20:11$). Farrell & Stufkens (1993) reported that *N. huttoni* showed a significant preponderance of males in ground samples ($1418\text{♀} : 1679\text{♂} = 1:1.18$, $P < 0.001$) and in suction trap collections ($524\text{♀} : 686\text{♂} = 1:1.31$, $P < 0.001$), but not in a vane trap ($421\text{♀} : 457\text{♂} = 1:1.09$, $P > 0.05$).

McDonald & Smith (1988) investigated the sex ratio of *Nysius vinitor* collected on most of their sampling occasions and the results showed a preponderance of males ($2430\text{♀} : 2970\text{♂} = 1:1.22$, $P < 0.05$, χ^2 test). This is in agreement with the results of some samples of *N. huttoni* collected from the field (Table 4-7). The sex ratios of *Nabis alternatus* Parshley and *N. americanoferus* Carayon (Stoner *et al.* 1975) were reported to be ca. 1:1 although the females had a slight edge (*N. alternatus* females 52.6%, males 47.3%; *N. americanoferus* females 51.1%, males 48.9%); and the numbers of males and females fluctuated as the population fluctuated in size. Rajasekhara & Chatterji (1970) reported that the ratio of females to males of *Orius indicus* in a laboratory

population was 1.60:1.00. In samples collected from the field, it was 1.20:1.00, indicating a preponderance of females over males in both cases. Working with the tarnished plant bug, *Lygus lineolaris*, Ridgway & Gyrisco (1960) reported that males (n = 661) significantly outnumbered females (n = 585) in collections made with a sweep net throughout the season ($\text{♀}:\text{♂} = 1:1.13$, $P < 0.05$, χ^2 test). This ratio is very close to that for *N. huttoni* averaged over the growing seasons of 1995-1996 and 1996-1997.

The sex ratio of *N. huttoni* adults in the field was close to 1:1 at most times. All samples that differed significantly from the expected 1:1 ratio had a male bias ($P < 0.05$ or 0.01) and came from the overwintered, second- or third-generation populations. The stable 1:1 sex ratio of first-generation adults in the field is the one that dominates species dynamics.

The reason why the proportion of males in some of the samples exceeded the expected 1:1 ratio is not clear, but the following factors may be involved.

One explanation for a sex ratio shift in insects is the difference in adult lifespan between the sexes. In Chapter 3, it was shown that mean adult longevity of males and females was similar at 15-30°C ($P > 0.05$), but females lived longer than males at 35°C ($P < 0.01$, Table 3-18). Mortality of adult males was higher than that of females at higher temperatures of 36-40°C (Table 3-20). These results indicate that temperatures of $\geq 35^\circ\text{C}$ could cause a decrease in the proportion of males in the adult population. However, in all three years, adult sex ratios were consistently male-biased in samples whose differences in proportion of females to males were statistically significant. Therefore, this explanation can not apply to *N. huttoni*.

A second possible explanation is that the species has an intrinsic male-biased sex ratio. However, the data in Table 4-4 show that the sex ratio of newly

emerged adults is 1:1. Furthermore, the sex ratios of adults produced at different temperatures indicate that the intrinsic sex ratio is 1:1.

A third possibility is that the dates of eclosion could differ between the sexes. This would result in a bias toward the earlier-eclosing sex early in the season, and, in time, a shift toward the later-eclosing sex. Male eggs of *Aedes triseriatus* hatch more readily than females from the same egg batch (Shroyer & Craig 1981). In the laboratory, I observed that males hatched a little earlier than females when eggs were exposed to the same temperature, although the developmental duration did not show significant differences between females and males ($P > 0.05$, Table 3-4). However, a difference in egg hatching behaviour between males and females may account for the seasonally male-biased sex ratio of newly hatched nymphs.

A fourth explanation is that migration into and out of the census area could differ between the sexes. Thus, either female emigration or male immigration would result in increasing male bias. In all three years of my study, adult sex ratios in all field samples of the first generation were stable with a sex ratio of 1:1. No one sample was found to have a male-biased sex ratio ($P < 0.05$). In the second generation, however, two samples in 1995-1996 and one in 1996-1997 produced a male-biased sex ratio ($P < 0.05$ and 0.01). When those samples were taken, the second-generation adults were in the peaks of eclosion and occurrence in the field. During that time and a short period earlier, the host plants in the habitats were dry and dead. Flight of adults took place when the weather was very hot. For example, most of the plants in the habitats had died when the flight of adults took place on 4 February 1996 when ground temperature reached 40°C and air temperature was 28°C at two o'clock in the afternoon. So, a possible explanation for the male-biased sex ratio may be a behavioural difference between males and females. The females may have migrated out of the habitat to reproduce, whereas males remained in the habitat where they emerged. This could also help explain the

stability of the sex ratio of the first generation because the host plants were still green at that time.

The fifth possible explanation is that photoperiod may have an effect on the sex ratio. The data in Table 4-6 show that short days may increase the proportion of males in the adult population, especially at lower temperatures. This may help explain why some adult samples of the third and overwintered generations produced male-biased sex ratios. Eclosion of third-generation adults started between late summer (February) and early autumn (March) and eclosion lasted until the end of autumn. In Christchurch, 12-h photophase occurs about 20 March and then the days become shorter. Therefore, the male-biased sex ratios in some adult samples from the third generation may have been associated with the shortening of the days. In contrast, stability of the sex ratio in the first generation was associated with development under a long-day photoperiod.

The sixth and final explanation for a sex-ratio shift is that sampling error may be a cause of bias. Further experimentation is required to elucidate why proportions of males and females in some samples may depart from 1:1.

4.3.6 One copulation fertilises a female for life

Table 4-8 shows the results of tests in which *N. huttoni* females were fertilised once. Of 14 female adults tested, one (No. 2) was dissected to check whether eggs were present after 20 days without oviposition. No mature eggs were found, so this female was omitted from further analyses. Another two females (Nos. 11 and 12) both lived 62 days but did not produce eggs for unknown reasons. Their lifespans were included in longevity calculations. The remaining 11 adults, which laid eggs during the test were divided into two types, according to whether or not all of the eggs they laid hatched.

Table 4-8. Results of tests to determine whether one copulation of *N. huttoni* can fertilise a female for life. The tests were carried out at 25°C and 12L:12D.

Adult no.	Date of emergence of adult	Date of copulation	Pre-oviposition period (in days)	Oviposition period (in days)			Post-oviposition period (in days)	Adult longevity (in days)	Total no. eggs tested					Max days between copulation and last viable egg
				Fertile period	Sterile period	Total			In fertile period			In sterile period	Total	
									No.laid	No.hatched	Hatching percentage (%)			
A: Results of adults that did not have sterile periods.														
3	19/12/97	20/12/97	56	32	0	32	0	88	45	32	71.1	0	45	87
5	19/12/97	20/12/97	15	28	0	28	0	43	41	29	70.7	0	41	42
6	19/12/97	20/12/97	15	7	0	7	2	24	13	13	100.0	0	13	21
8	19/12/97	20/12/97	15	18	0	18	1	34	22	15	68.2	0	22	32
9	19/12/97	20/12/97	15	23	0	23	0	38	26	24	92.3	0	26	37
14	15/01/98	2/02/98	17	18	0	18	0	35	33	22	66.7	0	33	34
Mean	---	---	22.2	21.0	0.0	21.0	0.5	43.7	30.0	22.5	78.2	0.0	30.0	42.2
Range	---	---	15-56	7-32	0-0	7-32	0-2	24-88	13-45	13-32	66.7-100.0	0-0	13-45	21-87
B: Results of adults that had sterile periods.														
1	19/12/97	20/12/97	40	20	28	48	0	88	34	23	67.6	23	57	59
4	19/12/97	20/12/97	60	10	18	28	0	88	9	6	66.7	6	15	69
7	19/12/97	20/12/97	35	25	28	53	0	88	46	25	54.3	30	76	59
10	15/01/98	19/01/98	4	44	57	101	4	109	60	39	65.0	31	91	47
13	15/01/98	19/01/98	4	34	92	126	26	156	50	38	76.0	30	80	37
Mean	---	---	28.6	26.6	44.6	71.2	6.0	105.8	39.8	26.2	65.9	24.0	63.8	54.2
Range	---	---	4-60	10-44	18-92	28-126	0-26	88-156	9-60	6-39	54.3-76.0	6-31	15-91	37-69
C: Results of adults that did not lay eggs.														
2*	19/12/97	20/12/97	---	---	---	---	---	---	---	---	---	---	---	Dissection
11**	15/01/98	19/01/98	---	---	---	---	---	62	---	---	---	---	---	No oviposition
12**	15/01/98	19/01/98	---	---	---	---	---	62	---	---	---	---	---	No oviposition
D: Total results of all adults tested.														
Total mean	---	---	25.1	23.5	20.3	43.8	3.0	70.4	34.5	24.2	70.2	10.9	45.4	47.6
Total range	---	---	4-60	7-44	0-92	7-126	0-26	24-156	9-60	6-39	54.3-100.0	0-31	13-91	21-87

*No. 2 was dissected to check whether it had eggs after living 20 days without laying; ** No. 11 and 12 both lived 62 days without laying and their lifespans were included in the mean longevity.

Type 1: females that did not have sterile periods

This type included six females (Table 4-8 A) in which some or all eggs from all batches hatched. This indicated there were no sterile periods during the oviposition period. The mean pre-oviposition period for these adults was 22.2 days (range 15-56 days); the oviposition period was 21 days (range 7-32 days); the post-oviposition period was 0.5 day (range 0-2 days); and adult mean longevity was 43.7 days (range 24-88 days).

The mean number of eggs tested for each female was 30 (13-45) and on average 22.5 of them hatched. Eggs of each individual female were shown to be fertile, and the mean hatching percentage was 78.2% (range 66.7 to 100%). This was lower than the hatching success of eggs produced by females with multiple copulations, as shown in Tables 3-2 and 3-3 (see Chapter 3). The mean viable life of sperm from copulation to producing the last batch of fertilised eggs was 42.2 days (range 21-87 days).

Type 2: females that had sterile periods

Five females belonged to type 2 (Table 4-8 B). In this type, each female showed a distinct fertile period in the early stage of oviposition and a sterile period in the late stage of oviposition. The mean fertile period for five females was 26.6 days (range 10-40 days) during which some or all eggs from each batch hatched. A total of 199 eggs from five females (mean 40 eggs per female) was tested and 131 of them hatched with a mean hatching rate of 65.9% (54.3-76.0%). The sterile period averaged 44.6 days (range 18-92 days) during which a total of 120 eggs (mean 24, range 6-31) produced by the five females was tested. None of these eggs produced nymphs. This suggests that all eggs laid during the sterile period were unfertilised.

The mean pre-oviposition period, oviposition period, post-oviposition period, and mean adult longevity of females of type 2 were longer than those of females of type 1. The mean percentage of eggs that hatched during the fertile

period in type 2 (65.9%) was lower than that of eggs in type 1 (78.2%), however. The mean viable longevity of sperm (54.2 days) of type 2 was about 12 days longer than that of type 1 (42.2 days). Sperm were found to live for 37-69 days.

In type 2 females, the mean fertile period (26.6 days) was about 18 days shorter than the mean sterile period (44.6 days). These two periods were 37.4% (27-47.2%) and 62.6% (52.8-73%), respectively, of the total mean oviposition period (71.2 days). Therefore, if a female copulates three times, she can produce fertilised eggs throughout her life.

Rajasekhara & Chatterji (1970) reported that one successful mating of *Orius indicus*, which had an oviposition period of 12 to 23 days was sufficient to fertilise the average number of eggs laid by a female in her lifetime. Similarly, Strong *et al.* (1970) reported that a single mating of *Lygus hesperus* Knight, which had a mean oviposition period of 23.5 days (range 12 to 34 days), was sufficient to produce viable eggs for her egg-laying life. However, Cusson *et al.* (1998) who studied the mating behaviour of *Tranosema rostrale rostrale* (Brishke) (Hymenoptera: Ichneumonidae) suggested that under laboratory conditions, a single mating may not be sufficient to provide a female with an adequate supply of sperm.

Spermatozoa evidently can be stored by the female of *N. huttoni* for long periods of time. No. 3 of type 1 and No. 4 of type 2 (Table 4-8) present typical examples. Both of them emerged on 19 December and copulated the following day. Their preoviposition periods were about two months long (56 days for No. 3 and 60 days for No. 4), that is, they did not laid eggs until the 55th and 59th day, respectively after copulation. No. 3 had an oviposition period of 32 days, during which 32 of 45 tested eggs hatched. This female died on the same day that she laid her last batch of eggs, indicating that the spermatozoa could be stored by her for 87 days. For No. 4, the oviposition period was 28 days, of

which the fertile period was 10 days during which 6 of 9 tested eggs hatched. Towards the end of the fertile period she started to lay unfertilised eggs and continued to do so until she died. Thus, No. 4 could store spermatozoa for 69 days. Although the preoviposition periods of these two females were longer than those of others, my results indicate that sperm were stored from copulation to the end of the fertile period.

The difference in longevity of females at different temperatures and photoperiods is considerable among individuals even if the adults are kept under the same conditions of temperature, photoperiod, food, and humidity (Tables 3-18 and 3-21, Chapter 3). Comparable differences in longevity presumably also exist in field populations. The results presented here show that if female longevity is about 1.5 months and the mean oviposition period is about three weeks, a single copulation may fertilise a female for life (Table 4-8 A). However, if mean longevity is 3.5 months and the mean oviposition period is about 10 weeks, a single copulation will fertilise a female for only part of her oviposition period (Table 4-8 B).

Eggs laid by females of type 2 during the sterile period did not hatch. There may be three possible reasons for this. The first is that the amount of sperm acquired by females is limited and a single copulation may not provide sufficient sperm to completely inseminate a female. The size of the chitinised spermathecae undoubtedly determines the amount of sperm retained by females. It is presumed that by the end of the fertile period, the sperm may have been used up by females. The second possible reason is that sperm may have lost their vitality completely towards the end of the fertile period. The third possible explanation is that hatching of eggs may be related to the age of the females and the hatching percentage of eggs may decrease gradually to zero in the end. Knight, Strong *et al.* (1970) studied the effectiveness of a single mating in a group of 18 females of *Lygus hesperus*. They found a significant change in the hatching percentage of eggs as the females matured.

For the first 15 or so days after mating, about 50% of the eggs hatched. From day 23 on, however, only about 12% hatched, and after day 30, none hatched.

N. huttoni is a multiple-mating species. In the field, a female can mate repeatedly with different males, and in the laboratory, copulation was repeatedly performed. Frequency of copulation has a bearing on the percentage of hatching of eggs since multiple mating can increase the hatching percentage of eggs. Hatching percentages of eggs laid by females with multiple mating (Tables 3-2 and 3-3, Chapter 3) were higher than those of eggs laid by females, which only mated once (Table 4-8).

Conclusions that can be drawn from this test are: (1) a single copulation is not sufficient to ensure fertilised eggs throughout the entire life of a female; (2) the hatching percentage of the fertilised eggs resulting from one insemination will be lower than that of fertilised eggs produced by multiple copulations; and (3) sperm can maintain vitality for one to three months.

4.3.7 Parthenogenesis

Results of tests undertaken to determine whether parthenogenesis occurs in *N. huttoni* are given in Table 4-9. A total of 35 unfertilised eggs laid by five virgin females in 1996, and 62 by five virgin females in 1997, were tested. None hatched. I conclude that parthenogenesis does not occur in *N. huttoni*, although further tests on more populations and under a greater variety of conditions are needed.

4.3.8 Seasonal variation of population density

Seasonal population trend of adults and nymphs in the field in 1995-1996 and 1996-1997 are shown in Figs. 4-3 C and 4-4 C. Fig. 4-3 C represents 23616 individuals (20459 nymphs and 3157 adults) from 28 samples collected from 5

September 1995 to 5 June 1996. Fig. 4-4 C is based on 18355 individuals (15133 nymphs and 3222 adults) from 22 samples collected from 5 September 1996 to 5 April 1997.

Table 4-9. Results of tests undertaken to determine whether parthenogenesis occurs in *N. huttoni*.

Adult no.	Date of emergence	Date of oviposition	No. eggs laid for test	No.hatched.
1996				
1	26/02/96	10/03/96	4	0
2	26/02/96	17/03/96	10	0
3	29/02/96	10/03/96	4	0
4	1/03/96	12/03/96	5	0
5	3/03/96	15/03/96	12	0
Total	-----	-----	35	0
1997				
1	19/12/97	25/12/97	15	0
2	19/12/97	24/12/97	16	0
3	19/12/97	28/12/97	8	0
4	19/12/97	25/12/97	12	0
5	19/12/97	24/12/97	11	0
Total	-----	-----	62	0

In 1995-1996, adults emerged from the overwintering site in early September. Only adults were in the field in September and population density was low throughout September. Numbers increased in October as first-generation nymphs appeared and peaked around 25 October. Then, the population began to decrease, reaching its lowest level in mid-November. When nymphs of the second generation appeared at the beginning of December, numbers again increased until the second peak appeared in early January. The second peak receded sharply around 10 January and reached its lowest level in late

January. In early February, nymphs of the third generation began to appear. The third peak in numbers occurred in late February. Numbers fell in early March and in mid-April population density was at its lowest.

In 1996-1997, population density of overwintered adults was low throughout September as it was in 1995-1996. The appearance of first-generation nymphs resulted in increasing in numbers in October as it did in 1995-1996. The first peak, however, occurred about 20 days later than it did in 1995-1996 and appeared in mid-November. Population density reached its lowest level in mid-December after the first peak. A second peak in numbers occurred in mid-January and was about 10 days later than in 1995-1996. The third peak, which occurred in mid-February appeared about 10 days earlier than it did in 1995-1996, however. Due to overlap between the second and third generations, there was no apparent period during which population density reached a very low level between the second and third peaks. In 1996-1997, insects disappeared from the field earlier than in 1995-1996.

Population structure indicated that three generations could be distinguished in the 1995-96 season, but not 1996-97. In both seasons, but especially 1996-97, 1st instar nymphs appeared in the field three times. Each appearance seems to be best interpreted as the beginning of a new generation rather than a consequence of delayed egg development and of coincident emergence brought about by climatic factors.

On this basis *N. huttoni* follows a distinct seasonal pattern: three peaks in numbers, representing three generations, occur in some of the years studied. When a new generation appears, the population begins to build up until a peak appears and then starts to decline to a very low level until the next generation occurs. Due to differences in development associated with weather conditions, the time of appearance of the three peaks in population numbers can differ between years.

Field observations indicate that population density is substantially influenced by host plants, and variability in population numbers is closely associated with the seasonal availability of host plants. When the weather is so hot that host plants become dry and die, mortality of nymphs is also high. Flights of adults to fresh habitats then occur, resulting in population density declining to a very low level. For example, during the last two weeks of November 1995, from mid-January to early February 1996, and during the whole of December 1996, most host plants in the study habitat at Hornby had died. Adult flight occurred at this time and the population of *N. huttoni* was greatly reduced.

Rainfall can also cause high mortality in *N. huttoni*, especially that of 1-3-instar nymphs. For example, heavy rainfall on 11 and 12 October 1998 resulted in a decline in size of the 1-2-instar nymphal population of the first generation. A large number of nymphs and adults were also killed by three days of rainfall on 11-13 March 1999.

CHAPTER FIVE

**Tolerance of adults to
starvation and effect of
glucose and water on adult
longevity**

Chapter 5

Tolerance of adults to starvation and effect of glucose and water on adult longevity

5.1 Introduction

Tolerance of starvation plays an important role in the life processes of insects and there have been studies related to this for many hemipterans (e.g., Panizzi & Hirose 1995a, 1995b; Ito 1984, 1986; Steinbauer 1998). However, no data have been reported on the effects of starvation on *N. huttoni*. The present study was aimed to rectify this situation to some extent. Differences in the ability of adults to resist starvation may result from differences in the amount of nutrients transferred from the nymphal stage, and therefore, may be reflected in differences in the nutritional base existing in the field at various times.

“Many of the life processes of insects require the expenditure of a significant amount of water. Among these are respiration, excretion, secretion, growth, and reproduction. Other vital activities recycle quantities of water that are required for satisfactory maintenance of metabolic functions” (Wharton 1985). Insects usually acquire water from their food, in which it can vary from 1 to over 90%. Despite its abundance in growing plant tissues, water can affect insect growth rates and adult longevity (Barbehenn *et al.* 1999, Panizzi & Hirose 1995a). It is not clear if, and for how long afterwards water alone can prolong the life span of *N. huttoni*. One aim of the present study, therefore, was to test the effect of water alone on the longevity of adults.

Insects feed on a wide range of plants, animals and dead organic matter. Their dietary requirements include proteins, amino acids, carbohydrates, lipids, vitamins and inorganic nutrients that are required for general growth and

metabolism as well as specialized reproductive and behavioural needs (Schowalter 2000). Like other animals, insects need proteins and/or other sources of amino acids to synthesize their own proteins, while carbohydrates, lipids and amino acids are used for metabolism (Barbehenn 1999, Wolersberger 2000, Ryan & van der Horst 2000).

Carbohydrates provide the main sources of energy for most insects (Olson *et al.* 2000) and are essential for optimal growth of many herbivorous larvae (Barbehenn 1999). Furthermore, to achieve maximum longevity, the adults of some other insect species require sugar meals (Tsiropoulos 1980a, 1980b, Chapman 1982, Jacob & Evans 2000). The ability of an insect to use a particular carbohydrate depends on a complex series of factors. Glucose, fructose and sucrose are nutritionally adequate sugars for most insects, but the ability to metabolize other dietary carbohydrates varies among species, and also may depend on the age, sex and metamorphic stage of the insect (Rockstein 1978). Some carbohydrates are nutritionally ineffective because they are hydrolysed or absorbed incompletely, whereas others are readily absorbed but cannot be metabolized. They may even inhibit enzymatic reactions, or act as feeding deterrents if present in high concentrations (Rockstein 1978).

Plants differ considerably in their nutritional value to insects, and the value of a particular species will often change seasonally or with the age of the plant (Schowalter 2000). Because *N. huttoni* is a polyphagous species that attacks over 40 diverse species of plant, including shepherds purse, twin cress, broom, lucerne, turnips, wheat and clover (Chapter 2), it can be expected to experience various combinations of carbohydrates in a variety of concentrations. However, whether they can all be used by *N. huttoni* is not known. The experiments described in this chapter tested the effect of a single carbohydrate (glucose) on the longevity of adults.

5.2 Materials and methods

All adults (both sexes) used in these experiments were newly emerged virgins at the beginning of each test.

Experiment 1. – Tolerance of starvation by adults of three generations.

The 70 adults (35 males and 35 females) used, emerged from 5th instar nymphs of the first, second, and third generations. Nymphs of the first generation were collected on 15 November, second generation on 23 December 1997, and third generation on 6 February 1998. Single adults were put in glass vials as described in Chapter 4, and kept at 20°C and 60-70% RH. No food was provided during the test. The numbers of dead adults were recorded daily.

Experiment 2. – Water, glucose and longevity. The purpose of this experiment was to test whether glucose or water alone could prolong the life of adults. The experimental insects used were adults that emerged from 5th instar nymphs of the third generation collected between 24 March and 15 April 1996. Ten experimental treatments were established: no food; water only; 2, 5, 10, 15, 20, 25, 30, and 40% glucose solutions made up in tap water. Seventy adults (35 males and 35 females) were used to test longevity in each treatment, each one being kept in a separate glass vial. The experiments were conducted at 20°C (12.5-29°C) and 60-70% RH, in the laboratory. In the water only treatment, water-soaked cotton wool was placed in each vial, whereas cotton wool soaked in glucose solution was placed in the third treatment. The vials were examined every 24 hours and the cotton wool plus water and glucose were renewed daily to prevent microbial growth. Numbers of dead adults were recorded daily.

Data obtained in Experiment 1 and 2 were analysed statistically using One-way ANOVA among treatments and with t-tests between males and females.

5.3 Results

Experiment 1. – Tolerance of starvation by adults of three generations.

Mean longevity of adults of the three generations is shown in Table 5-1. Non-feeding adults had short longevity after emergence, mean longevity being not more than 4 days. Differences in resistance to starvation were found between adults of the three generations. Mean starvation-longevity of first-generation adults (4.2 days for females, 4.1 days for males, and 4.2 days for both sexes combined) was significantly longer than that of second-generation adults (2.3 days for females, 2.1 days for males, and 2.2 days for both sexes combined) and third-generation adults (2.6 days for females, 2.4 days for males, and 2.5 days for both sexes combined) ($P < 0.05$, One-way ANOVA). However, no difference existed in mean starvation-longevity of second- and third-generation adults ($P > 0.05$).

Mean starvation-longevity between male and female adults within generations was not significantly different ($P > 0.05$, T-test), indicating that male and female adults have the same toleration to starvation under the same conditions.

Experiment 2. – Water, glucose and longevity. Only adults from the third generation were used to carry out experiments on the effects of water alone and glucose on adult longevity. Starvation-longevity of adults was also tested simultaneously with third-generation adults. Results are presented in Table 5-2 and Fig. 5.

When no food or water was provided after emergence, mean starvation-longevity was 4.7 days for females and 4.1 days for males (4.4 days for both sexes combined). No significant difference was found between sexes ($P > 0.05$, T-test). Longevity was therefore similar to that of first-generation adults as shown in Table 5-1 and provides support for the contention that the nutrient

Table 5-1. Mean starvation-longevities (in days \pm S.E.) of *N. huttoni* adults (ranges in parentheses).

sex	No. adults tested	1st generation	2nd generation	3rd generation
F	35	4.2 \pm 0.14a (3 - 6)	2.3 \pm 0.11b (1 - 3)	2.6 \pm 0.10b (2 - 4)
M	35	4.1 \pm 0.17a (2 - 6)	2.1 \pm 0.11b (1 - 3)	2.4 \pm 0.10b (1 - 3)
F + M	70	4.2 \pm 0.11a (2 - 6)	2.2 \pm 0.08b (1 - 3)	2.5 \pm 0.07b (1 - 4)

Means within a row followed by the same letter were not significantly different ($P > 0.05$; One-way ANOVA). Means for females and males within a generation were not significantly different ($P > 0.05$; T-test).

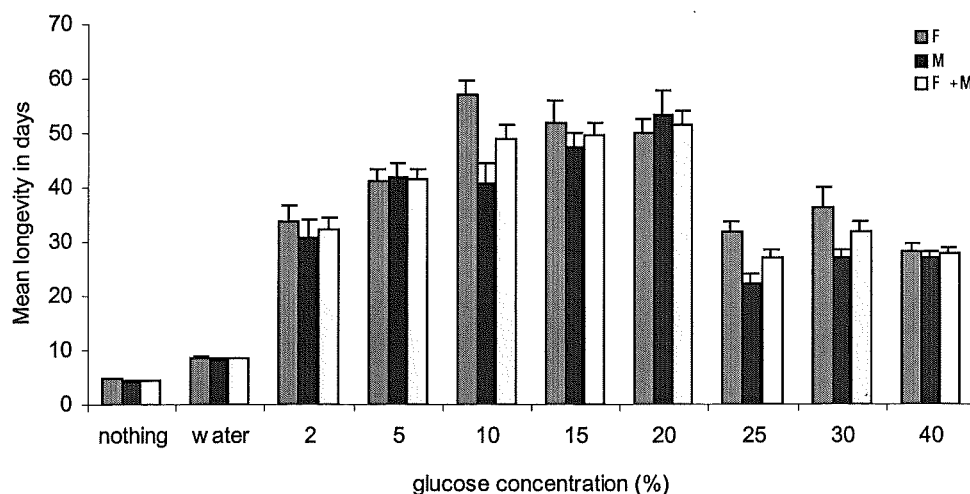
**Fig. 5.** Effects of water and glucose on adult longevity of *N. huttoni* (mean + S.E.)

Table 5-2. Effects of water and glucose on adult longevity (in days \pm S.E.) of *N. huttoni* (range in parentheses).

Sex	No. adults tested	Nothing	Water alone	Glucose (%)							
				2	5	10*	15	20	25*	30*	40
F	35	4.7 \pm 0.25f (3 - 8)	8.6 \pm 0.33e (4 - 12)	33.7 \pm 2.92cd (10 - 68)	41.1 \pm 2.36b (12 - 82)	57.1 \pm 2.61a (21 - 85)	51.9 \pm 3.86a (18 - 107)	49.9 \pm 2.83a (9 - 85)	31.8 \pm 2.04cd (11 - 59)	36.4 \pm 3.52c (7 - 79)	28.2 \pm 1.36d (13 - 45)
M	35	4.1 \pm 0.28g (3 - 11)	8.3 \pm 0.37f (4 - 14)	30.6 \pm 3.33d (7 - 76)	41.8 \pm 2.65c (18 - 87)	40.8 \pm 3.50c (8 - 74)	47.3 \pm 2.79b (13 - 90)	53.3 \pm 4.40a (10 - 109)	22.2 \pm 1.95e (7 - 51)	26.9 \pm 1.77de (9 - 58)	27.0 \pm 1.23de (8 - 43)
F + M	70	4.4 \pm 0.19f (3 - 11)	8.4 \pm 0.24e (4 - 14)	32.1 \pm 2.21c (7 - 76)	41.4 \pm 1.76b (12 - 87)	49.0 \pm 2.38a (8 - 85)	49.6 \pm 2.38a (13 - 107)	51.6 \pm 2.61a (9 - 109)	27.0 \pm 1.52d (7 - 59)	31.7 \pm 2.04cd (7 - 79)	27.6 \pm 0.91d (8 - 45)

Means within a row followed by the same letter were not significantly different ($P > 0.05$; One-way ANOVA). *Means for females and males at particular glucose concentrations were significantly different ($P < 0.05$; T-test).

status of nymphs influences adult longevity. Thus, host plants in the field had not dried and died when the nymphs of the third generation were collected for experiment 2, whereas they had when third generation nymphs were collected for experiment 1.

When water alone was provided for adults, starvation-longevity increased significantly ($P < 0.05$, One-way ANOVA). Thus, mean longevity was 8.6 days for females and 8.3 days for males, almost double that of adults given no food. No difference was found between sexes ($P > 0.05$, T-test).

Glucose in concentrations of 2-40% had a strong effect on adult longevity. In females, mean longevity increased significantly with glucose concentration between 2 and 10% ($P < 0.05$). Concentrations of 10, 15, and 20% gave the maximal mean longevity of adults (49.9-57.1 days) and differences in mean longevity among these three concentrations were not significantly different ($P > 0.05$). Mean longevity was significantly lower at 25, 30, and 40% ($P < 0.05$) than at 5, 10, 15, and 20%, however. Similarly in males, mean longevity increased significantly as glucose concentration increased from 2 to 20%. Maximum mean longevity (53.3 days) was obtained at the 20% concentration. Mean longevity at 25% (22.2 days), 30% (26.9 days), and 40% (27.0 days) concentrations were not significantly different from each other ($P > 0.05$) and were significantly lower than at 20% ($P < 0.05$).

Interestingly, females provided with 25, 30, and 40% glucose lived as long as those provided with 2% glucose, and the mean longevity of males at 30 and 40% glucose was not significantly different from that at 2% ($P > 0.05$). These results demonstrate that feeding on a carbohydrate with a concentration above the optimal concentration may result in comparable longevity to that attained when feeding on a low concentration of carbohydrate. The reason for this is not known, but reduction of longevity when provided with low-concentration sugar solutions may be the result of insufficient sugar content, whereas,

reduction in longevity at high concentrations of sugar may be due to the accumulation of excessive sugar by the insect.

No significant differences in mean longevity were found between the sexes at different sugar concentrations, except at 10, 25, and 30% when females lived longer than males ($P < 0.05$, T-test).

Differences were found in longevity among individuals at each concentration. For example, at 15%, female longevity ranged from 18 to 107 days, and at 20% male longevity ranged from 10 to 109 days. A possible reason for this may be different feeding histories of the nymphs, as found by Panizzi & Hirose (1995b) for the southern green stink bug, *Nezara viridula* (L.) whose adults lived significantly longer when nymphs were raised on sesame as opposed to soybean.

During my tests, no egg production occurred at any concentration of glucose no matter how long the female adults lived. This may have been because no protein was provided for oocyte development. The results, however, show that adults of *N. huttoni* can survive on a single carbohydrate diet (glucose).

5.4 Discussion

Adults of *N. huttoni* were able to withstand deprivation of food for an average 2-4 days after emergence. This indicates that upon emergence they have few stored nutrients. There are two types of starvation-longevity of adults. One is the starvation-longevity of non-feeding adults after adult emergence and the other is that of feeding adults after adult emergence. Differences in starvation-longevity between non-feeding and feeding adults may reflect physiological differences, which may be due to the accumulation of different amounts of nutrients before starvation. On the other hand, starvation-longevity of non-

feeding adults can reflect the nutritional history of the nymphal stage since nutrients accumulated in the nymphal stage can be used by the adult.

To obtain information on the relationship between availability of host plants in the field and survival capacity of adults, non-feeding adults tested immediately after emergence were used to examine tolerance to starvation. Thus, no additional nutrients were accumulated in the adult stage. Adults were supported only by nutrients accumulated in the nymphal stage and transferred to the adult upon emergence. The longevity of adults, therefore, should reflect the amount of nutrients accumulated from the nymphal stage.

My results show that the mean starvation-longevity of first-generation adults was about twice that of second- and third-generation adults. This suggests that the nutritional basis of first-generation adults was better than that of second- and third-generation adults. These findings are in agreement with field observations. When nymphs of the first generation were sampled on 15 November 1997, most of the plants in the field were still green and ample food plants were present. It would therefore be expected that nymphs could acquire sufficient nutrients for development. However, over 95% of food plants in the field had withered or died because of hot weather on 23 December 1997 and 6 February 1998 when nymphs of the second and third generations were collected. Differences in starvation-longevity of adults between generations are likely to be caused by the presence or absence of high quality food for nymphs in the field.

Ito (1986) studied starvation-longevity of adults of the coreid bug, *Cletus punctiger* under 12L:12D (short daylength condition) and 16L:8D (long daylength condition) at 25°C. He found that mean starvation-longevity of non-feeding bugs after adult emergence was 8-10 days. When bugs were fed for 5 days, starvation-longevity increased significantly; mean longevity under the long day regime was 20.4 days in females and 15.3 days in males, and 25.0

days in females and 21.9 days in males under the short day regime. Furthermore, under the short day regime, the longer the feeding period, the longer the starvation-longevity extended in both sexes up to 20-days. In *Nilaparvata lugens* (Hemiptera: Delphacidae), tolerance of starvation by macropterous adults was tested after 1.5, 3.5, and 6.5 days feeding. The 3.5-day feeding adults were found to have the highest tolerance (Kusakabe & Hirao 1976), although no reason for this was given.

Perhaps one of the most important effects of host plant availability on the nymphal stage of *N. huttoni* is its influence on the ability of adults to resist starvation after emergence. As stated by Panizzi & Hirose (1995), the ability of adults to resist starvation has major ecological implications, particularly at times of low food availability when energy is needed to disperse, to face periods of change in temperature, or similarly to sustain metabolism.

Water alone can extend the starvation-longevity of adults of some species of Hemiptera. For example, starvation-longevity of adults was 13.0-14.8 days in *Nezara viridula* and 24.6-34.6 days in the brown stink bug, *Euschistus heros* when supplied with water only, whereas it was less than 7 days for both species in the absence of water (Panizzi & Hirose 1995a).

Relative humidity was likely to have been one of the important factors operating in the experiment with water alone since it is known to have a significant effect on starvation-longevity of some adult insects. For example, the mean starvation-longevity of *Nysius vinitor* (Hehat & Wyndham 1972b) was significantly greater at 75 and 96% RH than at 32% RH at 20, 25, and 30°C. Starvation-longevity of *Amorbus obscuricornis* (Westwood) and *Gelonus tasmanicus* (Le Guillou) (Steinbauer 1998) was also significantly affected by relative humidity, being highest at 99 and 100% and lowest at 42, 63, and 79% at 14.3°C. Similar findings have been reported for other species including the

grasshopper, *Chortophage viridifasciata* (Ludwig 1937), and the Japanese beetle, *Popillia japonica* Newman (Ludwig & Landsman 1937).

Sugar is an important source of energy, and the importance of carbohydrate for survival has been shown for many insect species. Benschoter & Leal (1976) studied the effect of water only and sugar (sucrose, fructose, and glucose at concentrations of 5, 10, 20, and 40%) on longevity of the cotton leaf perforator, *Bucculatrix thurberiella* Busck. They found that mean longevity of adults increased with sugar concentration, but adult survival was less at a concentration of 5% compared with higher levels tested. Adults fed sugar lived 3 times longer (15.5 days) than those given nothing (5.2 days) or water only (5.0 days), but water did not extend the life span of adults compared to those receiving nothing. Tsiropoulos (1980a) investigated the nutritional requirements for survival of the adult olive fruit fly, *Dacus oleae* (Gmelin) using a chemically defined diet and found that the most important ingredient extending survival was sucrose. The same author (1980b) also studied carbohydrate utilisation by *Dacus oleae* offered 20% aqueous solutions of 23 carbohydrates. Males lived for an average of 53.5 days and females 45.3 days on 20% glucose, significantly longer than when offered water only (1.6 days for males and 1.4 days for females) and nothing (1.3 days for males and 1.0 days for females). Water did not extend the life span. These mean longevity values are very similar to those for *N. huttoni* when fed 20% glucose (Table 5-2).

Certain carbohydrates are toxic when fed to insects. Nettles (1972) studied the toxicity of sugars and longevity of *Anthonomus grandis* Boheman. In his work, longevity of sugar-fed adults was compared with that of water-fed ones to determine whether sugar was toxic. If the mean longevity of sugar-fed adults was shorter than that of water-fed ones, sugar was considered toxic. According to this criterion, glucose was not toxic to *N. huttoni* since the mean longevity of adults provided water alone in my experiments (8.4 days) was

shorter than that of adults at any glucose concentration tested (Table 5-1). My experiments also provided information on the amount of glucose required by adults to prolong their life spans. The optimal concentration for prolonging life was found to be 10-20%, at which adults of both sexes were able to live for about 50 days.

As noted above, *N. huttoni* can feed on as many as 40 species of wild and cultivated host plants. The different plant species contain different carbohydrates and the same carbohydrates will be present in different concentrations in different plant species. However, the actual concentrations of carbohydrates and concentrations of sugars are not known in all but one of the host plants used by *N. huttoni*. The exception is shepherd's purse, which was used for rearing *N. huttoni* in this research. Its main carbohydrates are sucrose, sorbose, lactose, amino-glucose, sorbitol, mannitol, and adonitol, and the total content of all carbohydrates in edible parts of the plant has been reported to be 4.8% (in dry weight) (the content of glucose is not available) (Jiangsu New Medical College 1985). This percentage is not very high but my results indicate it can partially meet the carbohydrate requirements of adults.

In my experiments with high concentrations of glucose (20-40%), mean longevity of adults declined. The reason for this is unclear. However, it is known that in the cockroach, *Periplaneta americana* (Treherne 1957) and the locust, *Schistocerca gregaria* (Treherne 1958, 1959), absorption of sugars is largely confined to the mid-gut caeca, and that absorbed sugars are converted, to varying degrees, to trehalose in the haemolymph. Treherne (1957) found that the rate of crop emptying was related to sugar concentration, so that the amount of fluid leaving the crop decreases with increasing concentration. This effect is determined by the osmotic pressure of the ingested fluid.

Two important limiting factors control the rate of sugar absorption (Rockstein 1978): the rate of release of fluid from the crop into the midgut, and the rate of conversion of absorbed glucose into trehalose. The first factor regulates the availability of glucose to the midgut epithelium. The control of fluid release from the crop into the midgut protects the midgut epithelium from being saturated by an excess of glucose. Neural or hormonal, or both pathways are involved in controlling the release of fluids from the crop.

The second factor regulates the rate of diffusion of glucose through the midgut epithelium by controlling the concentration gradient of glucose between the midgut lumen and the haemolymph. The synthesis and accumulation of haemolymph trehalose appear to play a central role in carbohydrate absorption by maintaining a deep concentration gradient of glucose between the intestinal lumen and the haemolymph, thereby facilitating glucose uptake by the physical process of diffusion. After monosaccharides, most commonly glucose and fructose, have been absorbed by the epithelial cells and transferred to the haemolymph, they are then transported to the fat body for trehalose synthesis. Trehalose is then released into haemolymph. This results in the maintenance of a high trehalose and low glucose concentration in the haemolymph that, in turn, facilitates glucose absorption by maintaining a steep concentration gradient between the intestinal lumen and the haemolymph. In addition, the presence of an active α -trehalase in the midgut cells appears to limit back-diffusion and loss of trehalose into the gut lumen, and to aid in maintaining a favourable concentration gradient for glucose absorption. Glucose is absorbed more rapidly than other monosaccharides because the fat body converts it into trehalose at a higher rate.

As sugar concentration increases, at least four important parameters are increased simultaneously. These are viscosity, nutritive value, stimulation power, and osmotic pressure. These variables were examined singly by Gelperin (1966) in X-ray photography studies of the control of crop emptying in

the blowfly, *Phormia regina*. He found that increasing the osmotic pressure of the solution in the crop slowed crop emptying, while increases in viscosity, nutritional value, and stimulating power did not influence the rate of crop emptying. He also found that increasing the osmotic pressure of the blood greatly slowed crop emptying. Since both blood osmotic pressure and the osmotic pressure of the ingested solution are important in crop emptying, the question is, which is the controlling one. Gelperin confirmed experimentally that the solute concentration of the blood apparently is the critical parameter. The ingested solution exerts its effect by increasing the solute concentration of the blood. He, therefore, concluded that the more concentrated the sugar solution ingested by the insect, the more slowly the crop will empty, i.e., a dilute sugar solution increases blood-solute concentration more slowly than a concentrated sugar solution and hence the dilute solution leaves the crop more rapidly.

A possible reason for the reduction in longevity of adult *N. huttoni* fed higher concentrations of glucose therefore may be the accumulation of excess glucose in haemolymph. At higher concentrations (20-40%), the osmotic pressure of the sugar solution in the gut will be greater than at lower concentrations. Increased osmotic pressure would then be expected to slow the rate of crop emptying. As noted above, the ingestion of high-concentration glucose solution may result in an increase in blood osmotic pressure, thus resulting in an accumulation of glucose in the haemolymph. The presence of appreciable amounts of glucose in the haemolymph would tend to reduce the concentration gradient between intestinal lumen and the haemolymph so net percentage absorption will be reduced.

High concentrations of glucose might also inhibit enzymatic reactions, including those involved in glycolysis and gluconeogenesis (Rockstein 1978). Nayar & Sauerman (1971) noted that carbohydrate must be metabolised in

association with the appropriate enzymes and that the rate of carbohydrate metabolism will be affected by the amount of enzyme present.

In summary, my study has indicated that starvation-longevity of adults of *N. huttoni* belonging to three generations was affected by the presence or absence of food in the nymphal stage in the field. The provision of water extended the starvation-longevity of adults, while life span was increased substantially when adults were fed glucose. Mean maximum longevity was obtained at 10-20% concentrations and declined at higher (25-40%) and lower (2-15%) concentrations.

CHAPTER SIX

Habits of *N. huttoni*

Chapter 6

Habits of *N. huttoni*

6.1 Introduction

Behaviour is shown by an organism as it interacts with, and adjusts to, its environment. Behaviour includes a very wide range of activities, and it can be helpful to recognise some subcategories (Matthews & Matthews 1982). Animals are able to develop their responses to stimuli, and in this way behaviours can be regarded as adaptive mechanisms (Manning 1979). Most insect behaviours are considered to be especially strongly influenced by heredity (Gullan & Cranston 2000), but they are also influenced by environmental factors (McFarland 1985). Insects, like other organisms, show great diversity in their behaviours. The behavioural subcategories of locomotion, dispersal, communication, defence, feeding, courtship, mating, oviposition, feigning death, and phototaxis are behavioural phenomena that occur in many species of insects (e.g., Hoikkala & Crossley 2000, Miyatake 2000, Belmain *et al.* 2000, Matsumoto & Sakai 2000, and Hochuli 2001). Some of these phenomena can be understood as direct responses to environmental changes. However, many more are strongly influenced by intrinsic factors, or events occurring within the animal (Saunders 1982).

Insects in their natural environment usually have strong adaptability to their particular circumstances (McFarland 1985). Study on behaviour of any particular insect species is important for better understanding of its biology and ecology. For example, many species of insects exhibit aggregative behaviour. Examples include the green stink bug, *Nezara viridula* (Hemiptera: Pentatomidae) (Todd 1989, Lockwood & Stoty 1986), the European corn borer, *Ostrinia nubilalis* (Showers *et al.* 2001), the ant, *Solenopsis richteri* Forel (Hymenoptera: Formicidae) (Wuellner 2000), the fly, *Crataerina melbae* (Diptera: Hippoboscidae) (Tella & Jovani 2000), and the strawberry blossom

weevil, *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) (Innocenzi *et al.* 2001). An understanding of the factors driving the distribution of an aggregating species within its habitat requires detailed knowledge of the scale, location and persistence of the aggregations (Thomas *et al.* 2001).

Eyles (1965b), Gurr (1952, 1957), and Farrell & Stufkens (1993) reported on some aspects of the biology, ecology and phenology of *N. huttoni*, including distribution, host plants, habitats, seasonal movements, life history, courtship behaviour and copulation (reviewed in detail in Chapter 1). However, many aspects of its general biology and habits remain unknown. During my work on the effects of different temperatures and photoperiods on oviposition and adult longevity, some empty chorions were found by chance on the surface of cotton wool that was provided for females to lay eggs on in the laboratory. This finding aroused my interest in examining whether cannibalism on eggs occurs. When field-collected bugs were sorted in the laboratory, both nymphs and adults showed positive phototaxis. Therefore, in this part of my research, experiments were conducted to determine whether *N. huttoni* fed on eggs and whether field population showed positive phototaxis. Also, in the course of my life-history work, some changes in the behavioural patterns of nymphs and adults were noticed. This led to my investigating letisimulation (feigning of death), aggregation, and mating behaviour.

6.2 Cannibalism on eggs

6.2.1 Materials and methods

The main aims of this experiment were to determine: (1) whether nymphs and adults (both sexes) will feed on eggs of *N. huttoni*; and (2) the number of eggs fed upon by each bug per day. Because *N. huttoni* lays eggs in soil, this experiment utilised soil in laboratory conditions as close to that in natural situations as possible.

A plastic container 5 cm high by 4 cm diameter (as described in Chapter 3) was half-filled with finely sieved soil (300 μ mesh sieve). The soil surface was wetted and then allowed to dry naturally so that the dust-like soil solidified and eggs or empty chorions were unlikely to be buried by soil disturbed by bug activity. Fresh eggs, which measure 0.77 x 0.28 mm (Eyles 1960) and were laid on cotton wool in glass tubes by female adults, were put on to the soil surface of the container. Bugs used in the feeding tests were nymphs and adults of the second generation, collected from the field. Nymphs were 3rd and 4th instars and adults had already emerged, so their ages since ecdysis were unknown. They were supplied with shepherd's purse as food for 24 hours and then starved for 24 hours so they were at the same level of hunger before experiments began.

Ten eggs and one bug were placed in each container. Two treatments were set up for nymphs, female adults, and male adults (total of 6 treatments). In one treatment, the insect was provided with food similar to that occurring in the field, while in the other, food was not provided. Twenty replicates of each treatment were set up. The experiment was conducted at about 25°C with 60% relative humidity at the beginning of January 1998. After 24 hours, numbers of eggs eaten were recorded.

6.2.2 Results and discussion

Number of bugs that fed on eggs within 24 hours. Results are given in Table 6. In treatments without plants, 70% of nymphs, 55% of females, and 65% of males fed on eggs within 24 hours. In treatments with plants, 65, 55, and 85% of nymphs, females and males ate eggs. No difference in egg mortality was detected ($P > 0.05$, U-test) between treatments with and without food for either nymphs, females or males. These results demonstrate clearly that *N. huttoni* will feed on its own eggs, and that both nymphs and adults of

Table 6. Numbers of *N. huttoni* cannibalising eggs in the laboratory.

Insect stage	Treatment	Replication	No. insects which fed on eggs in 24 hours			Eggs taken over 24 hours			Mean of eggs taken per feeding insect			Mean for all insects			Range
			No.	%	U-test	No.	%	U-test	Mean \pm S.E.	One-way Anova	T-test	Mean \pm S.E.	One-way Anova	T-test	
Nymph	1 nymph + 10 eggs	20	14	70.0	$P > 0.05$	75	37.5	$P < 0.01$	5.4 ± 1.0	abc	$P < 0.05$	3.8 ± 0.9	b	$P > 0.05$	0 - 10
	1 nymph + 10 eggs + plant	20	13	65.0		107	53.5		8.2 ± 0.9	ab		5.4 ± 1.1	ab		0 - 10
Adult	1 female + 10 eggs	20	11	55.0	$P > 0.05$	50	25.0	$P > 0.05$	4.5 ± 1.0	bc	$P > 0.05$	2.5 ± 0.7	b	$P > 0.05$	0 - 10
	1 female + 10 eggs + plant	20	11	55.0		42	21.0		3.8 ± 1.1	c		2.1 ± 0.7	b		0 - 10
	1 male + 10 eggs	20	13	65.0	$P > 0.05$	52	26.0	$P < 0.01$	4.0 ± 0.6	c	$P < 0.01$	2.6 ± 0.6	b	$P < 0.01$	0 - 8
	1 male + 10 eggs + plant	20	17	85.0		144	72.0		8.5 ± 0.6	a		7.2 ± 0.9	a		0 - 10

1. U-test was used to compare the percentage of insects that fed on eggs and percentage of eggs taken by insects over 24 hours between two treatments with and without plant in the same insect stage or sex and the results are shown in the table.

2. The differences in the mean number of eggs taken per insect among all treatments for feeding insects and for all insects were tested using One-way ANOVA. Means within a column shown by the same letter are not significantly different ($P > 0.05$).

3. T-test was performed in comparison of means between two treatments with and without plant in the same insect stage or sex and the results are shown in the table.

4. T-test was used to compare means between female and male treatments. For both feeding insects and all insects, the difference is significant ($P < 0.01$) for the treatments with plant, but not significant ($P > 0.05$) for the treatments without plant.

both sexes do so. The results also demonstrated that the presence or absence of an alternative plant food did not influence the number of bugs that feed on eggs. Egg-feeding behaviour would seem to be a characteristic behaviour of *N. huttoni*.

Number of eggs eaten in 24 hours. A total of 200 eggs was offered to bugs in the 20 replicates of each treatment. More eggs were eaten in the treatments with alternative foods than in those without food (53.5 vs 37.5% for nymphs and 72.0 vs 26.0% for males; $P < 0.01$, U-test) except in the case of females where no difference was found (25.0 vs 21.0%, $P > 0.05$).

The mean number of eggs eaten per bug (including those that did not feed) ranged from 2.1 to 7.2 per 24 hours in the 6 treatments (Table 6). No significant difference was found ($P > 0.05$, T-test) in the number of eggs taken per insect between treatments with and without food for nymphs (3.8 vs 5.4) and adult females (2.5 vs 2.1). However, the number of eggs taken per adult male was significantly greater ($P < 0.01$) when other food was provided (7.2 eggs/♂) than when no other food was given (2.6 eggs/♂). The reason for this is not clear. When no food was given, males fed on as many eggs (2.6/♂) as did females (2.5/♀) within 24 hours ($P > 0.05$, T-test), but males fed on more eggs (7.2/♂) than females (2.1/♀) when alternative food was offered ($P < 0.01$). My results indicate that overall the presence or absence of food has little effect on the number of eggs eaten by *N. huttoni*.

Observations on egg-feeding by a 2nd-instar nymph were made on 18 December 1995. Before starting to feed, the nymph cleaned its mouthparts (proboscis was repeatedly brushed by the fore legs) and then it put its fore legs onto the egg surface and pressed it down or held it to prevent it from moving in the process of feeding. It took the nymph seven minutes to complete cleaning its mouthparts and less than one minute to hold down the egg. The nymph then thrust its stylet into one end of the egg, and started to suck fluid

from it. When the fluid available at this end apparently became less, the nymph raised the other end of the egg with its legs to make the fluid flow to the end being sucked. Meanwhile, in the process of sucking, the stylet was vibrating. Egg contents were completely sucked out in 32 minutes between entry and withdrawal of the stylet. After this nymph had finished feeding on one egg, it started to feed on another one. On another occasion, two 1st-instar nymphs were seen feeding on the same egg at the same time.

The experimental results and observations given here show that nymphs and adults of *N. huttoni* cannibalise eggs. These results, however, were obtained in the laboratory and egg-feeding has yet to be confirmed in the field.

Adult females can feed on eggs laid by themselves and by other females. In the experiment discussed here, the eggs used were not their own, but in the process of rearing adults I observed females feeding on eggs laid by themselves in glass tubes. This indicates that females do not necessarily feed selectively on eggs laid by other individuals. In studies on the biology of two other hemipteran species, *Rhopalimorpha obscura* White and *R. lineolaris* Pendergrast (Pentatomidae), Pendergrast (1952) reported that a starved male *R. obscura* was observed sucking the contents from three partially incubated eggs of *R. lineolaris*, and that 15 eggs in a jar with starved individuals of both species were found with their contents sucked out. I did not look for feeding on the eggs of other insect species.

Rajasekhara & Chatterji (1970) reported that older nymphs of *Orius indicus* sometimes attacked younger nymphs. Similarly, Stewart (1968) observed that second and third instar nymphs of *Lygus rugulipennis* Poppius (Hemiptera: Miridae) were frequently seen sucking haemolymph from the bodies of siblings, which were especially vulnerable to attack while moulting. In the American cockroach, *Periplaneta americana* (Griffiths JR & Tauber, 1942), many females ate their own egg capsules, even though adequate food was

always present; some females also attacked and killed males. In *N. huttoni*, however, cannibalism of nymphs by older nymphs or by adults was not observed. Therefore, feeding on eggs by nymphs and adults is the only form of cannibalism known in this species. All previous publications on *N. huttoni* infer it is purely phytophagous, and mine is the first study to demonstrate a degree of carnivory (egg cannibalism) at least in the laboratory.

The reason why this species feeds on eggs is unknown. However, it appears that temperature and soil humidity may affect the occurrence of cannibalism, the number of insects that feed on eggs, and the number of eggs eaten by each insect. This was indicated by a simple test of the effect of temperature and humidity on cannibalising eggs by *N. huttoni* conducted with four treatments: damp soil at 20 and 30°C without plants, and dry soil at 30°C with and without plants. Each treatment consisted of one 4th-5th instar nymph and 5 eggs and was replicated 20 times. The numbers of eggs eaten were determined 24 hours later.

On damp soil, 10 (50%) out of 20 nymphs ate 28 (28%) of 100 eggs within 24 hours at 30°C and each nymph took on average 1.4 eggs. In contrast, at 20°C, only 4 (20%) nymphs ate eggs within 24 hours and only 6 (6%) eggs were eaten. Each nymph fed on an average of 0.3 egg per day. The difference between treatments was significant ($P < 0.05$, T-test) and indicated that the number of nymphs that took eggs, and the number of eggs eaten per nymph were greater at the higher temperature on damp soil.

On dry soil at 30°C, 13 (65%) nymphs ate eggs within 24 hours when no plant food was offered and 12 (60%) fed on eggs when food was available. Each nymph took on average 2.3 and 2.0 eggs per day in each treatment, means that were not significantly different ($P > 0.05$, T-test). The results of these experiments indicate that nymphs take more eggs under conditions of high temperature and dry soil than at low temperature and on damp soil. Feeding

on eggs may be one of the methods used by *N. huttoni* to supplement or gain water (as well as nutrition) under conditions of high temperature, low humidity, or both. Eggs may also be a source of protein required by nymphs for growth and by adults for reproductive development.

6.3 Phototaxis

6.3.1 Materials and methods

A blacklight trap (a 6 watt fluorescent tube) was constructed to attract *N. huttoni* at the research site. A white-painted wooden board with an area of 200 x 200 cm was placed vertically on the ground and the blacklight was hung horizontally against the middle of it. The light was run on two nights (27 and 28 November 1997), for two hours (2200-2400 h) when the weather was fine. The population in the field at the time consisted solely of adults of the first generation. Adults that had been collected during the previous day were also used to see whether they would respond to the light. These day-collected adults were put in an uncovered tray (30 x 40 cm), which was placed beside the board on the ground.

6.3.2 Results and discussion

Laboratory observations. Positive phototaxis of *N. huttoni* was noticed in the laboratory during 1995-1996 and 1996-1997. When working in the laboratory, I found that nymphs and adults, which were collected from the field and were put in a tray to be sorted or counted, moved towards the light produced by a 20 w fluorescent tube and gathered together in the corner of the tray or alongside it. The light tube was about 40 cm above the table surface. When the tray containing aggregated bugs was turned around, the bugs moved towards the light source again, and finally gathered together in another corner of the tray. The same procedure was repeated several times and the same

consequences were observed. These observations indicate that *N. huttoni* was positively phototaxic.

Field observations. In order to verify what had been observed in the laboratory, field tests were carried out on 27 and 28 November 1997. The results I obtained suggested that phototaxis was not very strong. When the light was turned on at 2200 h, the adults around the light moved towards the light source. However, they did not gather together, and many continued to wander about beneath the light. Over the two hours, the density of bugs became larger under the light, indicating they were attracted by the light.

When adults that had been collected the previous day were put into a tray located on the ground against the board, they behaved in a similar way to that seen in the laboratory. Thus, they moved to the side of the tray nearest the light. Although some returned to the other side of the tray away from the light or wandered inside the plate, most stayed in the area where there was most light.

During the two nights of tests, no flights of *N. huttoni* were observed. As will be discussed later, the flight of this species may be associated with temperature, which, at night, is too low to result in flight by adults.

Many species of Hemiptera have been reported to be positively phototaxic (Zhang shi-mei 1985). The phototaxis of some species is strong, e.g., *Geotomus pygmaeus* (Fabricius), *Plautia fimbriata* (Fabricius), *Physopelta cincticollis* Stål, and *Cyrtorrhinus lividipennis* Reuter, while phototaxis of *Erthesina fullo* (Thunberg), *Stollia guttiger* (Thunberg), and *Homoeocerus marginellus* Herrich-Schaffer has been described as weak. Species with moderately strong phototaxis include *Nezara viridula* (Linnaeus), *Liorhyssus hyalinus* (Fabricius), *Ectomocoris atrox* Stål, and *Nabis sinoferus* Hsiao.

6.4 Letisimulation, aggregation, and female-dragging movement in copulation

Methods, results and discussion

Letisimulation, aggregation characteristics, and mating behaviour were observed and investigated in the field and the laboratory during 1995-1999.

Many species of Hemiptera have been reported to possess letisimulation, including *Cressona valida* Dallas, *Picromerus griseus* (Dallas), *Dybowskyia reticulata* (Dallas), *Stollia ventralis* (Westwood), *Eurydema gebleri* Kolenati, *Urochela lutoovariva* Distant, *Nysius ericae* (Schilling), *Pyrrhocoris tibialis* Stål, and *Eteoneus angulatus* Drake et Maa (Zhang shi-mei 1985). Aggregation occurs commonly in hemipterans including *Paracopta duodecimpunctatum* (Germar), *Picromerus griseus* (Dallas), *Aelia nasuta* Wagner, *Halyomorpha halys* (Stål), *Urochela lutoovariva* Distant, *Lygaeus equestris* (Linnaeus) (Zhang shi-mei 1958), and *Oncopeltus fasciatus* (Dallas) (Sauer & Feir 1973).

6.4.1 Letisimulation

In the laboratory, letisimulation (feigning of death) was observed when nymphs were disturbed. This behaviour was first observed when nymphs and adults were being sorted in a tray. When disturbed, the nymphs contracted the legs, proboscis, and antennae, immediately and feigned death for about 2-5 seconds. This behaviour was more common in 1st and 2nd instar nymphs than 3rd-5th instar ones. Similar behaviour by nymphs was observed in the field. Adults did not exhibit this behaviour in the laboratory or field, however.

Death feigning is a common defensive behaviour in many insect species. In *N. huttoni*, it may afford nymphs some protection from being attacked. Also, like the protective coloration of other insects, the body colour of nymphs of *N.*

huttoni is very similar to sand particles, and they are very hard to distinguish from their environment when they are still. In the laboratory too, it was very hard to distinguish motionless nymphs from sand particles, especially 1st and 2nd instar nymphs, which were very similar to sand particles in both colouration and size.

Ambrose (1999) stated that the nymphal camouflaging of assassin bugs can be: (a) natural camouflaging, which denotes camouflaging by the natural colour of the body or with sand particles, foliage fragments, exuviae, egg shell, molluscan shells and other debris, or (b) corpse camouflaging, which denotes camouflaging by arenaceous materials as well as the empty “cases” of their prey. Nymphs of *N. huttoni* clearly have the former type of camouflaging.

Although predation of *N. huttoni* by other predators was not observed in the field, feigning death on disturbance should favour their survival.

6.4.2 Female-dragging movement in copulation

Eyles (1965b) described the mating behaviour of *N. huttoni* in detail, but did not describe the behaviour considered here. After a male and a female have achieved connection in the dorsal-lateral position, the act of copulation is usually shifted to an end-to-end position. I noticed that when the adults in copula started to move, the female dragged the male backwards at both walking and running speeds. This movement by the female continued until the male was finally displaced. During copulation, no other locomotory movements were seen: the female only walked forwards, whereas the male may actively walk backwards, or allow himself to be dragged along passively.

In the laboratory and the field, I made attempts to change this female-dragging movement into male-dragging movement by preventing the female's walking, using tweezers or a small stick. All attempts failed. The female would rather

turn right or left many times, or go back where she came from, than change and go backwards dragged by the male. Even if the female was forced into a corner by the tweezers or two sticks forming an angle, she preferred to stop walking rather than be dragged by the male. A total of 56 pairs in the laboratory and 125 pairs in the field were tested in the same way, but no pair changed to male-dragging movement.

A "partial exception" found was where a female in copula was dead and the male walked forwards. To test the generality of this male response, 23 paired females in the laboratory, and 72 paired females in the field were killed. All males walked forward dragging the dead females backwards.

Female-dragging behaviour in copula may be associated with the body size of adults of *N. huttoni*. Thus, in 260 pairs of adults observed in copula females were always bigger than their partners. This size difference may indicate that females are stronger than males and therefore pull them along.

Similar behaviour has been observed in *Anthocoris sarothamni* Douglas (Hemiptera: Anthocoridae) (Hill 1961), which walks carrying the male with her in copulation and by Malipatil (1979) in a study of 24 Australian species of Lygaeidae. Malipatil stated that once female and male adults go into the usual end-to-end copulation, the female drags the smaller male. When disturbed, the copulating pair tries to run away for shelter, often in the direction of the female, which drags the male after her. If a 'safe' shelter is unavailable and the disturbance continues, they immediately separate and run away in different directions.

6.4.3 Aggregation characteristics

Aggregation was observed primarily in the laboratory, and was shown by both nymphs and adults. When being sorted in the laboratory, bugs gathered

together in large number in the corner of the tray. If the population was big, they piled up, or formed clusters, or lined up along the sides of the tray. In the field, bugs were observed to aggregate in similar ways to those seen in the laboratory, under or around plants, debris, and clods of earth.

This behaviour may be associated with changes in ambient temperature. In a natural environment, insects may react to fluctuating temperatures by moving to different areas (Sauer & Feir 1973). Barret & Chiang (1967a, 1967b), who studied aggregations of 50 nymphs of *Oncopeltus fasciatus* (Hemiptera: Lygaeidae) at heat spots in cages at 70% relative humidity, found that the percentage that aggregate, and the actual temperature chosen, were different in each of three intrainstar periods. If the ambient temperature was dropped from 30 to 15°C, most nymphs moved to the heat spots but ecdysis never occurred on the heat spots.

In summary, laboratory experiments and observations showed that nymphs and adults of both sexes of *N. huttoni* can feed on eggs. The presence or absence of plant food did not influence the number of bugs that fed on eggs and had little effect on the number of eggs eaten by *N. huttoni*. Laboratory observations indicated that *N. huttoni* showed positive phototaxis. However, field tests indicated the phototaxis was not very strong. Nymphs but not adults exhibited letisimulation when disturbed. Nymphs and adults showed aggregation. During copulation in the end-to-end position, adults always displayed female-dragging behaviour that may be associated with the body size.

CHAPTER SEVEN

**Wing polymorphism and flight
in *N. huttoni***

Chapter 7

Wing polymorphism and flight in *N. huttoni*.

7.1 Introduction

Wing polymorphism is common among insects (Chapman 1982, Harrison 1980). It plays an important role in the life cycle of many insects and is usually viewed as an example of dispersal polymorphism (Harrison 1980). Wing polymorphism occurs in many insects, especially species of Orthoptera (e.g., Zera & Larsen 2001, Zera *et al.* 2001), Coleoptera (e.g., Carter 1976, Lloyd 1999), Homoptera (e.g., Johnson & Birks 1960, Byrne 1990), and Hemiptera (e.g., Fujisaki 1989a, 1989b, Sakashita *et al.* 1995, 1996). Within a species, individuals with fully developed wings (macropters) are generally capable of flight, while those either with reduced wings (brachypters or micropters) or without wings lack flight capability, but achieve enhanced reproductive abilities (Zera & Denno 1997).

Wing polymorphism has long been of interest in studies of a variety of important aspects of evolutionary biology such as the evolution of dispersal (Harrison 1980, Fairbairn & Desranleau 1987, Zera & Denno 1997), the evolution of life-history traits (Roff 1986, Solbreck 1986, Zera *et al.* 2001), and the evolution of the developmental program (Kodet & Nielson 1980, Braune 1983, Applebaum & Heifetz 1999, Zera & Huang 1999, Zera *et al.* 2001). There are often morphological differences associated with different flight behaviour (Harrison 1980). It is now widely accepted that wing polymorphism is one of the more important ways in which insects respond to environmental variations because dispersal ability can affect a species' ability to track resources in time and space (Denno *et al.* 1981).

It has been demonstrated in a wide variety of insect species that wing development is strongly influenced by environmental factors, including abiotic

factors such as temperature (Honek 1976b, Honek 1981, Aukema 1990, Sakashita *et al.* 1995) and photoperiod (Honek 1976a, Hardie 1987, Vaz-Nunes & Hardie 1996) as well as biotic factors such as food resources (Aukema 1990, Novotny 1994) and density of conspecifics (Applebaum & Heifetz 1999). It could be that the mechanism by which these environmental factors are affecting wing polymorphism is by altering levels of juvenile hormone in the developing embryos or larvae.

The modification of wings is a fairly common phenomenon in the Lygaeidae and occurs frequently in many, if not most, other families of Hemiptera (Slater 1975). A striking feature of the New Zealand insect fauna is that wing reduction is common and results in secondary flightlessness. Examples are known in New Zealand species of Phasmatodea, Acrididae, Dermaptera, Blattodea, Plecoptera, and in members of several families of Hemiptera, Lepidoptera, Diptera, Hymenoptera, and Coleoptera, despite fully winged forms being the normal condition in these insect orders (Watt 1975). Malipatil (1977) noted that the New Zealand ground-living lygaeid fauna is composed almost entirely of members of the tribe Targaremini and that 95% of the species are flightless, with coleoptery (hardening of wings) being the predominant condition. He observed high proportions of flightlessness in several other orders of New Zealand insects. Eyles (1960) noted that *N. huttoni* appears to be unique amongst the Orsillini in occurring in three wing forms in both sexes: macropterous, sub-brachypterous, and brachypterous.

An understanding of the ecology of species within the Lygaeidae is important in determining the selective factors responsible for wing polymorphism (Slater (1975). Wing polymorphism has been reported in many species of Hemiptera, including *Pyrrhocoris sibiricus* (Sakashita *et al.* 1995, 1996), *Cavelerius saccharivorus* Okajima (Fujisaki 1989a, 1989b), *Leptopterna dolabrata* (Braune 1983), *Jadera aeola* (Tanaka & Wolda 1987), and *Pyrrhocoris apterus* (Honěk 1976a, 1976b, and 1981). Wing polymorphism and flight of *N. huttoni*,

however, are poorly understood, especially those factors affecting wing length and its effect on flight.

The objective of my study was (1) to survey the proportion of each wing form in a field population, (2) to survey the proportion of copulating pairs among three wing forms, (3) to determine whether temperature and photoperiod affect wing polymorphism, and (4) to observe and investigate flight activity and environmental factors affecting flight in the field.

7.2 Materials and methods

7.2.1 Wing polymorphism

Proportion of each wing form in a field population. As no data have been reported in previous papers that show the proportions of various wing forms in natural populations of *N. huttoni*, a field survey was undertaken. Adults were collected from the field to determine the percentage of each wing form. Sampling was carried out for three years from 1995-1996 to 1997-1998 with collections being made at 10-day intervals on the 5th, 15th, and 25th of each month starting from September of the year in which the overwintered adults emerged (except 1995-1996) and ending in April or May of the next year when adults of the third generation disappeared for overwintering. The collected adults were measured and graded into three wing forms according to the criteria of Eyles (1960). These forms are: (1) Macropterous (M), in which the wings extend beyond the apex of the abdomen; (2) Sub-brachypterous (Sb), in which the posterior tips of the wings are level, or scarcely exceed, the apex of the abdomen; and (3) Brachypterous (B), in which the posterior of the wings does not reach the posterior of the abdomen.

Measurements were made while the adults were still alive, since the abdomen can shorten after adults die and become dry to some extent. In the process of

measuring wing length, some misclassification can occur since the three wing forms were identified by comparing whether or not the wing was longer than, shorter than, or level with the apex of the abdomen. The abdomens of females become swollen towards oviposition, thus changing wing length relative to the length of the abdomen as they develop. Therefore, females whose wings were originally a little bit longer than the abdomen were easily classed as the Sb-form. Misclassification of females for this reason may be one reason why the proportion of females in the Sb-form is greater than that of males. The extent of the fat-body in both sexes made the abdomen of the adults swollen, also resulting in changing the wing/abdomen length relationship. This could possibly have increased the proportion designated Sb in both sexes as well.

Proportions of copulating pairs among three wing forms in the field population. Theoretically, there are nine possible mating combinations among three wing forms. However, the proportion of each combination in the field population is unknown. When mating was at its peak, paired adults in copula were collected from the field and examined using the three wing length criteria described above. Collections were made in 1995-1996 and 1996-1997.

Effect of temperature and photoperiod on the development of wings. Measurements were made in conjunction with experiments on the effect of temperature and photoperiod on development of *N. huttoni* described in Chapter 3. Newly emerged adults were measured 48 hours after emergence, and the numbers of each form were recorded.

7.2.2 Flight

Temperature and flight. Initial field observations indicated that adults rarely flew, but did when temperatures were high. To determine the effect of temperature on flight, more observations and collections of flying adults were made from 6-27 February 1998 and from 18 February - 6 March 1999. During

these two periods, the weather in Christchurch was much hotter than usual (see results). Proportions of males and females, and numbers of each wing form were recorded to compare them with those of the ground adult population. Ground and air temperatures were recorded at the times that flights occurred.

Age and flight. Flying females collected in 1997-1998 and 1998-1999 were dissected to examine their degree of reproductive maturation so as to determine whether there was a relationship between age and flight. Nonflying females, which were collected from the ground population at the same time as flying adults, were also dissected so their maturation could be compared with that of the flying adults.

Starvation and flight. A test was carried out to see whether absence of food was one of the factors affecting flight. Adults were collected on 27 February 1998 and males and females were kept together to starve for 48 hours in the laboratory. The starved males and females were then separated and placed in trays to determine whether they would fly or not. The test was conducted for 15 minutes with each sex at 25°C in the laboratory. The numbers of bugs that took off and the numbers of adults that flew off the tray were recorded.

Sexual deprivation and flight. A test was carried out to determine whether flights of adults may be to find mates when proportions of males and females in the field differ considerably for some reason. Two hundred adults (100 males and 100 females) were collected from the field on 3 March 1998. The males and females were kept separately from each other for 48 hours with sufficient food provided. Sexually starved males and females were then put in separate trays and flight activity was recorded at 25°C in the laboratory. Tests lasted 15 minutes for each sex and numbers taking off and flying off the trays were recorded.

Light and flight. A test was conducted to determine whether flights of adults were associated with light differing in brightness. The test was carried out at 25°C in the laboratory, in the shade, and in full sunlight out of doors. Forty adults (20 males and 20 females) were collected from the field and were kept together in the laboratory for 24 hours with food offered. Then they were placed in a tray and flight activity was monitored: (1) in the laboratory for 15 minutes; (2) in the sunlight for 15 minutes; (3) in the shade for 15 minutes, and (4) in the sunlight again for another 15 minutes.

Statistical analysis. χ^2 tests were used to test the significance of differences in proportions of males and females in each wing form, flying and nonflying adults, differences in proportions of the mating combinations ♀M x ♂Sb and ♂M x ♀Sb in M x Sb, ♀M x ♂B and ♂M x ♀B in M x B, and ♀Sb x ♂B and ♂Sb x ♀B in Sb x B, and also differences in percentages of mating pairs between observed and expected mating combinations among the three wing forms. A U-test was used to compare the percentage of take-offs and the percentage of male and female insects that flew away from the testing tray in tests of the relationship between starvation and flight activity.

7.3 Results

7.3.1 Wing polymorphism

Proportion of each wing form in a field population. Table 7-1 and Fig. 7-1 show frequency distributions of each wing form in adults collected from a field population during three years from 1995-1998. Note that in 1995-1996, no collections were made between the beginning of September and mid-November, so data for overwintered adults and some first-generation adults are absent.

Table 7-1. Numbers and percentages of each wing form of *N. huttoni* adults collected from the field in three years, 1995-1998.

Sampling date	Sample size	Macropterous						Sub-brachypterous						Brachypterous						Generation
		No.			%			No.			%			No.			%			
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	
1995-1996																				
25/12/95	142	61	69	130	43.0	48.6	91.5	9	3	12	6.3	2.1	8.5	0	0	0	0.0	0.0	0.0	1
15/01/96	121	41	80	121	33.9	66.1	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	2
25/01/96	489	232	231	463	47.4	47.2	94.7	20	6	26	4.1	1.2	5.3	0	0	0	0.0	0.0	0.0	2
5/02/96	920	358	491	849	47.4	53.4	92.3	60	10	70	6.5	1.1	7.6	1	0	1	0.1	0.0	0.1	2
15/02/96	658	270	332	602	47.4	50.5	91.5	49	7	56	7.4	1.1	8.5	0	0	0	0.0	0.0	0.0	2
25/02/96	157	78	76	154	47.4	48.4	98.1	3	0	3	1.9	0.0	1.9	0	0	0	0.0	0.0	0.0	2
5/03/96	70	32	35	67	47.4	50.0	95.7	2	1	3	2.9	1.4	4.3	0	0	0	0.0	0.0	0.0	2
15/03/96	66	23	42	65	47.4	63.6	98.5	1	0	1	1.5	0.0	1.5	0	0	0	0.0	0.0	0.0	2 + 3
25/03/96	660	262	379	641	47.4	57.4	97.1	7	12	19	1.1	1.8	2.9	0	0	0	0.0	0.0	0.0	3
5/04/96	86	28	48	76	47.4	55.8	88.4	2	8	10	2.3	9.3	11.6	0	0	0	0.0	0.0	0.0	3
15/04/96	196	72	91	163	47.4	46.4	83.2	13	20	33	6.6	10.2	16.8	0	0	0	0.0	0.0	0.0	3
25/04/96	63	24	21	45	47.4	33.3	71.4	6	12	18	9.5	19.1	28.6	0	0	0	0.0	0.0	0.0	3
5/05/96	129	46	41	87	47.4	31.8	67.4	15	27	42	11.6	20.9	32.6	0	0	0	0.0	0.0	0.0	3
15/05/96	99	43	30	73	47.4	30.3	73.7	8	18	26	8.1	18.2	26.3	0	0	0	0.0	0.0	0.0	3
25/05/96	28	12	5	17	47.4	17.9	60.7	1	10	11	3.6	35.7	39.3	0	0	0	0.0	0.0	0.0	3
Total	3884	1582	1971	3553	47.4	50.7	91.5	196	134	330	5.0	3.5	8.5	1	0	1	0.0	0.0	0.0	----
1996-1997																				
5/09/96	274	111	123	234	40.5	44.9	85.4	23	17	40	8.4	6.2	14.6	0	0	0	0.0	0.0	0.0	Overwintered
15/09/96	167	79	67	146	47.3	40.1	87.4	16	4	20	9.6	2.4	12.0	1	0	1	0.6	0.0	0.6	Overwintered
25/09/96	751	282	381	663	37.5	50.7	88.3	54	33	87	7.2	4.4	11.6	1	0	1	0.1	0.0	0.1	Overwintered
5/10/96	575	220	240	460	38.3	41.7	80.0	54	46	100	9.4	8.0	17.4	12	3	15	2.1	0.5	2.6	Overwintered
15/11/96	476	215	253	468	45.2	53.2	98.3	3	5	8	0.6	1.1	1.7	0	0	0	0.0	0.0	0.0	1
25/11/96	437	207	218	425	47.4	49.9	97.3	8	4	12	1.8	0.9	2.7	0	0	0	0.0	0.0	0.0	1
5/12/96	663	331	317	648	49.9	47.8	97.7	12	3	15	1.8	0.5	2.3	0	0	0	0.0	0.0	0.0	1
15/12/96	244	122	113	235	50.0	46.3	96.3	3	6	9	1.2	2.5	3.7	0	0	0	0.0	0.0	0.0	1
25/12/96	200	93	97	190	46.5	48.5	95.0	7	3	10	3.5	1.5	5.0	0	0	0	0.0	0.0	0.0	1
5/01/97	465	210	220	430	45.2	47.3	92.5	21	10	31	4.5	2.2	6.7	2	2	4	0.4	0.4	0.9	1 + 2
15/01/97	440	203	229	432	46.1	52.0	98.2	3	5	8	0.7	1.1	1.8	0	0	0	0.0	0.0	0.0	2 + 1
25/01/97	400	183	209	392	45.8	52.3	98.0	2	4	6	0.5	1.0	1.5	1	1	2	0.3	0.3	0.5	2
5/02/97	200	105	92	197	52.5	46.0	98.5	1	2	3	0.5	1.0	1.5	0	0	0	0.0	0.0	0.0	2
15/02/97	200	94	103	197	47.0	51.5	98.5	0	2	2	0.0	1.0	1.0	0	1	1	0.0	0.5	0.5	2
25/02/97	200	93	105	198	46.5	52.5	99.0	1	1	2	0.5	0.5	1.0	0	0	0	0.0	0.0	0.0	2 + 3
5/03/97	200	84	114	198	42.0	57.0	99.0	1	1	2	0.5	0.5	1.0	0	0	0	0.0	0.0	0.0	3
15/03/97	200	105	91	196	52.5	45.5	98.0	1	3	4	0.5	1.5	2.0	0	0	0	0.0	0.0	0.0	3
25/03/97	200	78	114	192	39.0	57.0	96.0	1	4	5	0.5	2.0	2.5	0	3	3	0.0	1.5	1.5	3
5/04/97	200	85	108	193	42.5	54.0	96.5	2	3	5	1.0	1.5	2.5	1	1	2	0.5	0.5	1.0	3
Total	6492	2900	3194	6094	44.7	49.2	93.9	213	156	369	3.3	2.4	5.7	18	11	29	0.3	0.2	0.4	----

Table 7-1. Continued.

Sampling date	Sample size	Macropterous						Sub-brachypterous						Brachypterous						Generation
		No.			%			No.			%			No.			%			
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	
1997-1998																				
5/09/97	152	85	64	149	55.9	42.1	98.0	1	2	3	0.7	1.3	2.0	0	0	0	0.0	0.0	0.0	Overwintered
15/09/97	156	77	73	150	49.4	46.8	96.2	4	2	6	2.6	1.3	3.8	0	0	0	0.0	0.0	0.0	Overwintered
25/09/97	200	82	115	197	41.0	57.5	98.5	0	3	3	0.0	1.5	1.5	0	0	0	0.0	0.0	0.0	Overwintered
5/10/97	210	96	106	202	45.7	50.5	96.2	5	1	6	2.4	0.4	2.9	2	0	2	1.0	0.0	1.0	Overwintered
15/10/97	176	82	92	174	46.6	52.3	98.9	1	0	1	0.6	0.0	0.6	1	0	1	0.6	0.0	0.6	Overwintered
25/10/97	109	43	65	108	39.4	59.6	99.1	0	0	0	0.0	0.0	0.0	0	1	1	0.0	0.9	0.9	Overwintered
15/11/97	195	100	94	194	51.3	48.2	99.5	1	0	1	0.5	0.0	0.5	0	0	0	0.0	0.0	0.0	1
25/11/97	120	63	53	116	52.5	44.2	96.7	4	0	4	3.3	0.0	3.3	0	0	0	0.0	0.0	0.0	1
5/12/97	209	103	99	202	49.3	47.4	96.7	3	3	6	1.4	1.4	2.9	0	1	1	0.0	0.5	0.5	1
15/12/97	200	85	97	182	42.5	48.5	91.0	8	1	9	4.0	0.5	4.5	5	4	9	2.5	2.0	4.5	1
25/12/97	142	66	74	140	46.5	52.1	98.6	1	1	2	0.7	0.7	1.4	0	0	0	0.0	0.0	0.0	1 + 2
5/01/98	200	104	89	193	52.0	44.5	96.5	5	1	6	2.5	0.5	3.0	1	0	1	0.5	0.0	0.5	2 + 1
15/01/98	200	110	86	196	55.0	43.0	98.0	3	1	4	1.5	0.5	2.0	0	0	0	0.0	0.0	0.0	2
25/01/98	200	100	100	200	50.0	50.0	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	2
5/02/98	200	95	105	200	47.5	52.5	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	2 + 3
15/02/98	200	84	107	191	42.0	53.5	95.5	1	4	5	0.5	2.0	2.5	0	4	4	0.0	2.0	2.0	3
25/02/98	200	83	116	199	41.5	58.0	99.5	0	1	1	0.0	0.5	0.5	0	0	0	0.0	0.0	0.0	3
5/03/98	200	88	96	184	44.0	48.0	92.0	4	7	11	2.0	3.5	5.5	3	2	5	1.5	1.0	2.5	3
15/03/98	100	45	53	98	45.0	53.0	98.0	1	1	2	1.0	1.0	2.0	0	0	0	0.0	0.0	0.0	3
25/03/98	100	41	56	97	41.0	56.0	97.0	1	1	2	1.0	1.0	2.0	0	1	1	0.0	1.0	1.0	3
5/04/98	278	115	153	268	41.4	55.0	96.4	2	8	10	0.7	2.9	3.6	0	0	0	0.0	0.0	0.0	3
15/04/98	122	54	57	111	44.3	46.7	91.0	1	7	8	0.8	5.7	6.6	0	3	3	0.0	2.5	2.5	3
Total	3869	1801	1950	3751	46.5	50.4	97.0	46	44	90	1.2	1.1	2.3	12	16	28	0.3	0.4	0.7	-----

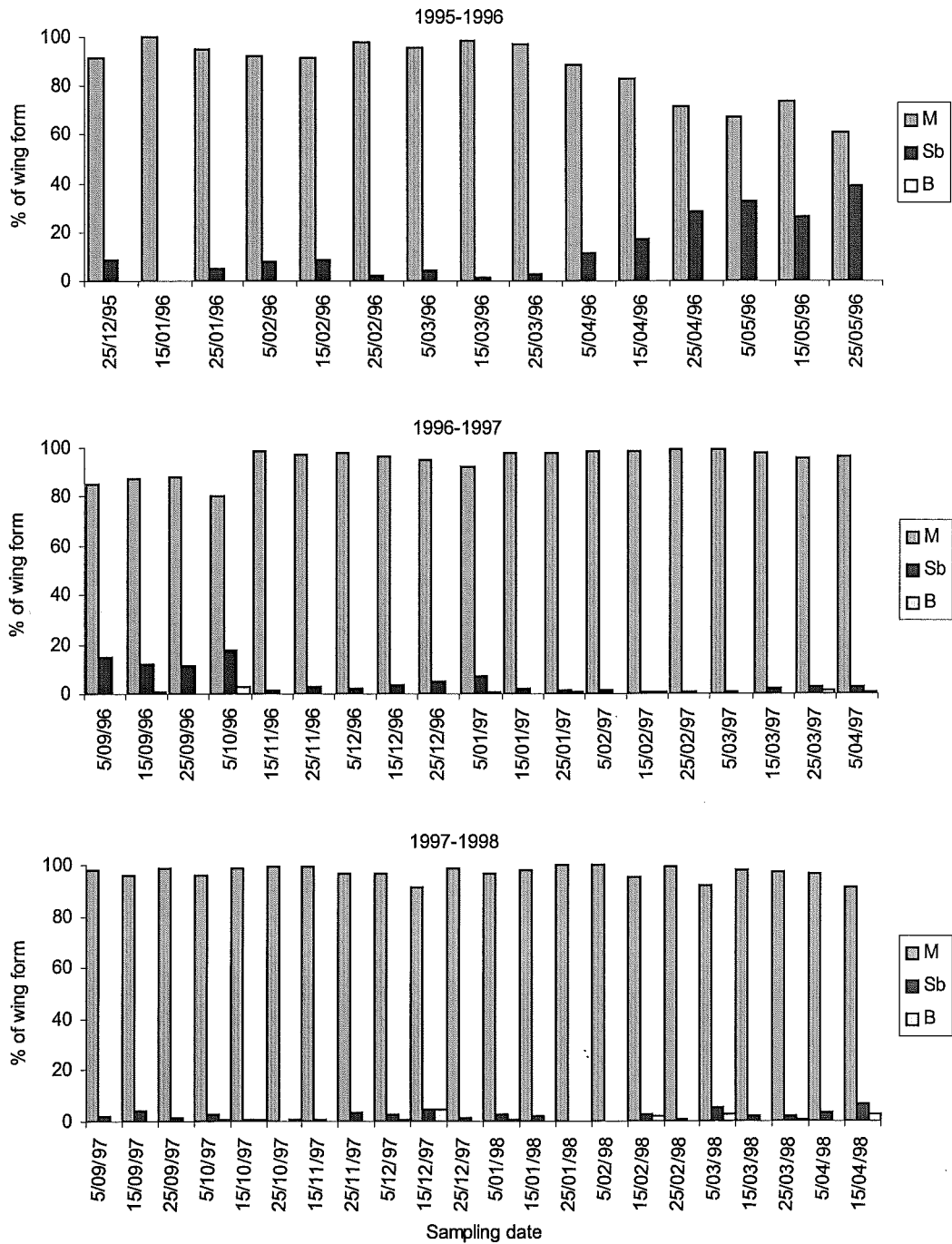


Fig. 7-1. Percentages of three wing forms of *N. huttoni* adults collected from the field in three years, 1995-1998. M - macropterous; Sb - sub-brachypterous; B - brachypterous.

In total, 14245 adults were taken in 56 samples and examined during the three years. There were large differences in percentages of the three wing forms, with the M-form being predominant (91.5% - 97.0% in the 3 years). Representation of the Sb-form ranged from 2.3% in 1997-1998 to 8.5% in 1995-1996, while the B-form was the rarest, not exceeding 1% of the population and ranging from 0% in 1995-1996 to 0.7% in 1997-1998.

Incidence of the M-form was greater in males than females ($P < 0.05$ or 0.01 , χ^2 test) with the sex ratio being 1:1.25 (♀:♂) in 1995-1996, 1:1.10 in 1996-1997, 1:1.08 in 1997-1998, and 1:1.13 for all three years combined (Table 7-2). The proportion of females was greater than that of males for the Sb-form ($P < 0.01$) with the sex ratio being 1:0.68 in 1995-1996, 1:0.73 in 1996-1997, 1:0.96 ($P > 0.05$) in 1997-1998, and 1:0.73 for all three years combined. Differences between females and males in proportions of the B-forms were not significant in the three years ($P > 0.05$).

In the field population, the M-form was predominant at all times during the three years, and the B-form varied between 0% and 2.6% of the population except for one sample in which it reached 4.5%. In contrast, the Sb-form in third-generation adults gradually increased in proportion from the beginning of April 1996 towards winter, and during early spring of the next season the proportion of wings represented by the Sb-form was higher than in any other season (Table 7-1 and Fig. 7-1). This trend was not evident before or after the winter of 1997 or before the winter of 1998 and may have been associated with the occurrence of third-generation adults. As shown in Fig. 4-1 and Table 4-2, third-generation adults occurred about 35 and 45 days later in 1996 (mid-March) than in 1997 (mid-February) and 1998 (very early February). This may have resulted in the occurrence of more Sb-forms in autumn 1996 than in autumn 1997 and 1998, because of lower temperature and shorter photoperiod. In summary, my results indicate that the Sb-form is mainly

Table 7-2. Sex ratios of the 3 wing forms of *N. huttoni* collected from the field in three years, 1995-1998.

Year	Macropterous				Sub-brachypterous				Brachypterous				Total sex ratio for three forms			
	♀+♂	♀	♂	♀:♂ (1:x)	♀+♂	♀	♂	♀:♂ (1:x)	♀+♂	♀	♂	♀:♂ (1:x)	♀+♂	♀	♂	♀:♂ (1:x)
1995-1996	3553	1582	1971	1:1.25**	330	196	134	1:0.68**	1	1	0	1:0.00	3884	1779	2105	1:1.18**
1996-1997	6094	2900	3194	1:1.10**	369	213	156	1:0.73**	29	18	11	1:0.61	6492	3131	3361	1:1.07**
1997-1998	3751	1801	1950	1:1.08*	90	46	44	1:0.96	28	12	16	1:1.33	3869	1859	2010	1:1.08*
Total	13398	6283	7115	1:1.13**	789	455	334	1:0.73**	58	31	27	1:0.87	14245	6769	7476	1:1.10**

Proportions of females and males measured were compared with the expected 1:1 (♀:♂) ratio using a chi-squared test. *P < 0.05, **P < 0.01.

associated with third-generation adults and the proportion found is likely to be associated with differences in weather among years.

Proportions of copulating pairs among three wing forms in the field population. Numbers and percentages of copulating pairs among different wing forms collected during 1995-1996 and 1996-1997 are given in Table 7-3. A total of 1414 pairs of adults in copula were collected and examined during the two years. As mentioned above, there are theoretically nine mating combinations among the three wing forms, however, only 6 were found in the field. Four mating combinations, M x M, ♀M x ♂Sb, ♂M x ♀Sb, and Sb x Sb occurred in both years; and two mating combinations, ♂M x ♀B and ♂Sb x ♀B were found only in 1996-1997. The other three mating combinations, ♀M x ♂B, ♀Sb x ♂B, and B x B were not found in either year.

M x M was the predominant combination in the field (Fig. 7-2), ranging from 78.8% in 1995-1996 to 82.5% in 1996-1997 (Table 7-3). The average percentage was 80.9% when total data from the two years were summed. The next most common mating combination was ♂M x ♀Sb with 11.4% in 1996-1997 and 16.8% in 1995-1996, an average of 13.7% for the two years combined. Proportions of the other 4 mating combinations, ♀M x ♂Sb, Sb x Sb, ♂M x ♀B, and ♂Sb x ♀B were very low and their overall percentages were 1.8-3.3% (mean 2.6%), 1.6-1.8% (mean 1.7%), 0.0-1.1% (mean 0.6%), and 0.0-0.1% (mean 0.1%), respectively.

Six mating combinations may occur in the M x Sb, M x B, and Sb x B pairs: ♀M x ♂Sb and ♂M x ♀Sb, ♀M x ♂B and ♂M x ♀B, and ♀Sb x ♂B and ♂Sb x ♀B in Sb x B. In M x Sb, the proportion of ♂M x ♀Sb (11.4-16.8%, mean 13.7% for two years) was higher than that of ♀M x ♂Sb (1.8-3.3%, mean 2.6%) ($P < 0.05$, χ^2 test). This follows the higher numbers of males than females in the M-form and of females than males in the Sb-form (Table 7-2). In M x B, the proportion of ♂M x ♀B (found only in 1996-1997) was 1.1%, but no

Table 7- 3. Percentages of different wing form combinations found in copula in the field, 1995-1996 and 1996-1997.

Sampling date	No. pairs in copula sampled	M* x M		M x Sb*				M x B*				Sb x Sb		Sb x B				B x B		Generation
				♀M x ♂Sb		♂M x ♀Sb		♀M x ♂B		♂M x ♀B				♀Sb x ♂B		♂Sb x ♀B				
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
1995 - 1996																				
24/12/95	42	30	71.4	3	7.1	9	21.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1
24/01/96	48	43	89.6	0	0.0	5	10.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2
2/02/96	127	73	57.5	3	2.4	43	33.9	0	0.0	0	0.0	8	6.3	0	0.0	0	0.0	0	0.0	2
10/02/96	139	102	73.4	0	0.0	31	22.3	0	0.0	0	0.0	6	4.3	0	0.0	0	0.0	0	0.0	2
14/02/96	99	89	89.9	1	1.0	9	9.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2
24/02/96	51	49	90.7	0	0.0	2	3.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2
Total	506	386	78.8	7	1.8	99	16.8	0	0.0	0	0.0	14	1.8	0	0.0	0	0.0	0	0.0	----
1996 - 1997																				
24/09/96	98	69	70.4	4	4.1	22	22.4	0	0.0	1	1.0	2	2.0	0	0.0	0	0.0	0	0.0	Overwintered
25/09/96	101	71	70.3	5	5.0	20	19.8	0	0.0	0	0.0	5	5.0	0	0.0	0	0.0	0	0.0	Overwintered
5/10/96	100	59	59.0	9	9.0	20	20.0	0	0.0	7	7.0	5	5.0	0	0.0	0	0.0	0	0.0	Overwintered
1/12/96	209	197	94.3	2	1.0	10	4.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1
22/12/96	100	90	90.0	3	3.0	7	7.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1
8/01/97	100	80	80.0	3	3.0	14	14.0	0	0.0	1	1.0	1	1.0	0	0.0	1	1.0	0	0.0	1 + 2
20/01/97	100	98	98.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2 + 1
27/01/97	100	98	98.0	0	0.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2
Total	908	762	82.5	27	3.3	96	11.4	0	0.0	9	1.1	13	1.6	0	0.0	1	0.1	0	0.0	----

*M - Macropterous; Sb - Sub-brachypterous; B - Brachypterous.

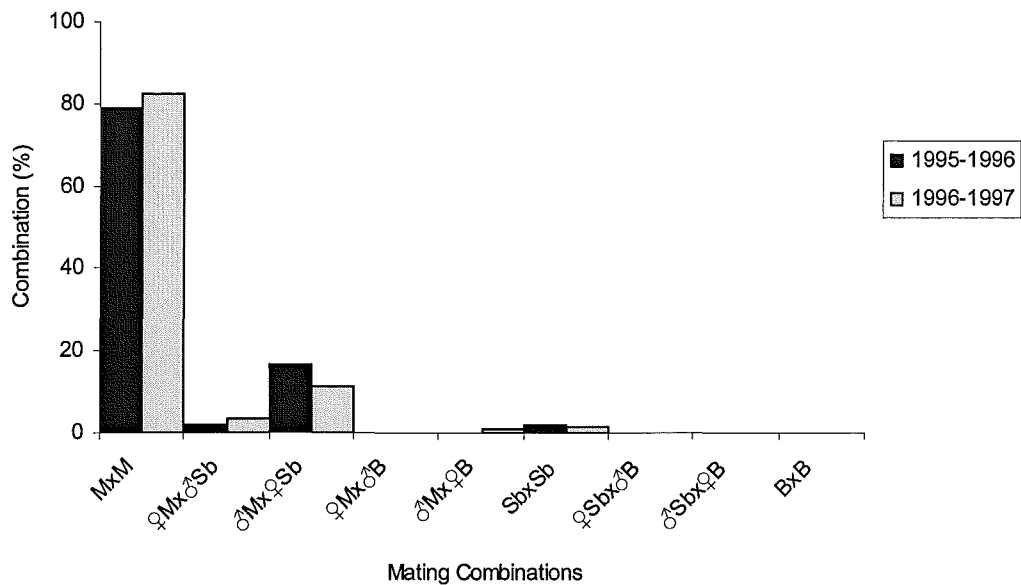


Fig. 7-2. Percentage of 9 wing form combinations of *N. huttoni* in copula in field populations during 1995-1996 and 1996-1997. M - macropterous; Sb - sub-brachypterous; B - brachypterous.

♀M x ♂B was found in either year. This result could be a higher proportion of males than females in the M-form. In Sb x B, however, only one pair of ♂Sb x ♀B was found in 1996-1997; no ♀Sb x ♂B was observed in either year.

The proportions of females and males of the three wing forms in 1995-1996 and 1996-1997 (Table 7-1) were used to calculate the expected percentage for each of the 9 mating combinations. The observed percentage of each mating combination (summed for the two year classes) was then compared with the expected one (Table 7-4). No significant difference was detected ($P > 0.05$, χ^2 test), indicating that no assortative mating occurred in the natural population.

Effect of temperature on the development of wings. Table 7-5 shows the number and percentage of each wing form produced at different temperatures under a 12L:12D photoperiod. At low temperature (15°C), no M-form was produced, but both Sb- and B-forms occurred. The proportions of these two

Table 7-4. Observed and expected percentages of mating pairs collected from the field during 1995-1996 and 1996-1997.

	M x M	M x Sb		M x B		Sb x Sb	Sb x B		B x B
		♀M x ♂Sb	♂M x ♀Sb	♀M x ♂B	♂M x ♀B		♀Sb x ♂B	♂Sb x ♀B	
Observed (%)	80.9	2.6	13.7	0.0	0.6	1.7	0.0	0.1	0.0
Expected (%)	86.3	4.8	7.8	0.2	0.4	0.4	0.0	0.0	0.0

$P > 0.05$, χ^2 test.

Table 7-5. Effect of temperature on wing development of *N. huttoni* in the laboratory.

Temperature (°C)	Total no. adults	Macropterous						Sub-brachypterous						Brachypterous					
		No.			%			No.			%			No.			%		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
15	19	0	0	0	0.0	0.0	0.0	6	3	9	31.6	15.8	47.4	4	6	10	21.1	31.6	52.6
20	81	31	26	57	38.3	32.1	70.4	2	15	17	2.5	18.5	21.0	2	5	7	2.5	6.2	8.6
25	47	17	20	37	36.2	42.6	78.7	4	6	10	8.5	12.8	21.3	0	0	0	0.0	0.0	0.0
30	111	41	30	71	36.9	27.0	64.0	15	20	35	13.5	18.0	31.5	1	4	5	0.9	3.6	4.5
35	212	61	95	156	28.8	44.8	73.6	26	9	35	12.3	4.3	16.5	16	5	21	7.5	2.4	9.9
L-T*	120	52	42	94	43.3	35.0	78.3	11	15	26	9.2	12.5	21.7	0	0	0	0.0	0.0	0.0

*L-T: Lab temperature with mean 20.3°C (12.5-29.5°C).

forms were 47.4% and 52.6%, respectively. As the temperature increased towards 25°C, the proportion of the B-form decreased to zero at 25°C, at which the proportion of the M-form was 78.7%. The proportion of the B-form increased again with further increases in temperature (Fig. 7-3). These results indicate that both low (15°C) and high (over 25°C) temperatures result in a reduction in the proportion of the M-form in the population. A similar effect of temperature on wing form was found in *Pyrrhocoris apterus* (Honek 1976a), in which the proportion of macropters was higher at intermediate temperatures (25-27°C) than at lower (about 21°C) and higher (over 30°C) temperatures. At lab temperature (L-T), the proportions of three wing forms of *N. huttoni* were the same as those at 25°C, suggesting that a fluctuating temperature is favourable for producing the M-form.

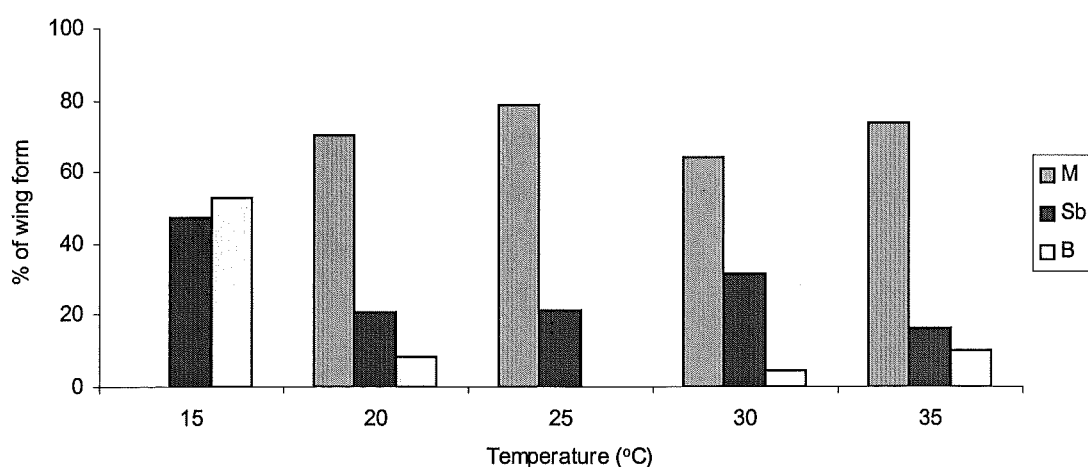


Fig. 7-3. Effect of temperature on wing form in *N. huttoni* as indicated by laboratory experiments. **M** - macropterous; **Sb** - sub-brachypterous; **B** - brachypterous.

Effect of photoperiod on the development of wings. Table 7-6 and Fig. 7-4 show the effects of three different photoperiods on wing development at 20°C. Under a 16:8 h photoperiod, no B-form occurred, the proportion of the Sb-form was very low (2.8%) and 97.2% of the population were of the M-form. Under a 12-h photoperiod, the proportion of the B-form was 8.6% and with further shortening of the photoperiod to 8 hours, it increased slightly more to 10%.

Table 7-6. Effect of photoperiod on wing development of *N. huttoni* in the laboratory.

Temperature (°C)	Photoperiod (L:D)	Total no. adults	Macropterous						Sub-brachypterous						Brachypterous					
			No.			%			No.			%			No.			%		
			♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
20	16 : 8	71	42	27	69	59.2	38.0	97.2	0	2	2	0.0	2.8	2.8	0	0	0	0.0	0.0	0.0
	12 : 12	81	31	26	57	38.3	32.1	70.4	2	15	17	2.5	18.5	21.0	2	5	7	2.5	6.2	8.6
	8 : 16	40	12	18	30	30.0	45.0	75.0	2	4	6	5.0	10.0	15.0	0	4	4	0.0	10.0	10.0
27.5	16 : 8	104	49	50	99	47.1	48.1	95.2	1	0	1	1.0	0.0	1.0	0	4	4	0.0	3.8	3.8
	12 : 12	112	41	71	112	36.6	63.4	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0
	8 : 16	92	46	43	89	50.0	46.7	96.7	0	2	2	0.0	2.2	2.2	0	1	1	0.0	1.1	1.1

The proportion of the Sb-form increased as well under 12-h and 8-h photoperiods to 21% and 15%, respectively. Correspondingly, the proportions of the M-form decreased to 70.4% and 75.0%. Thus, at 20°C, shortening of the photoperiod resulted in an increase in the B-form. A similar tendency was observed in the proportion of the Sb-form between 16-h and 12-h photoperiods but not between 12-h and 8-h.

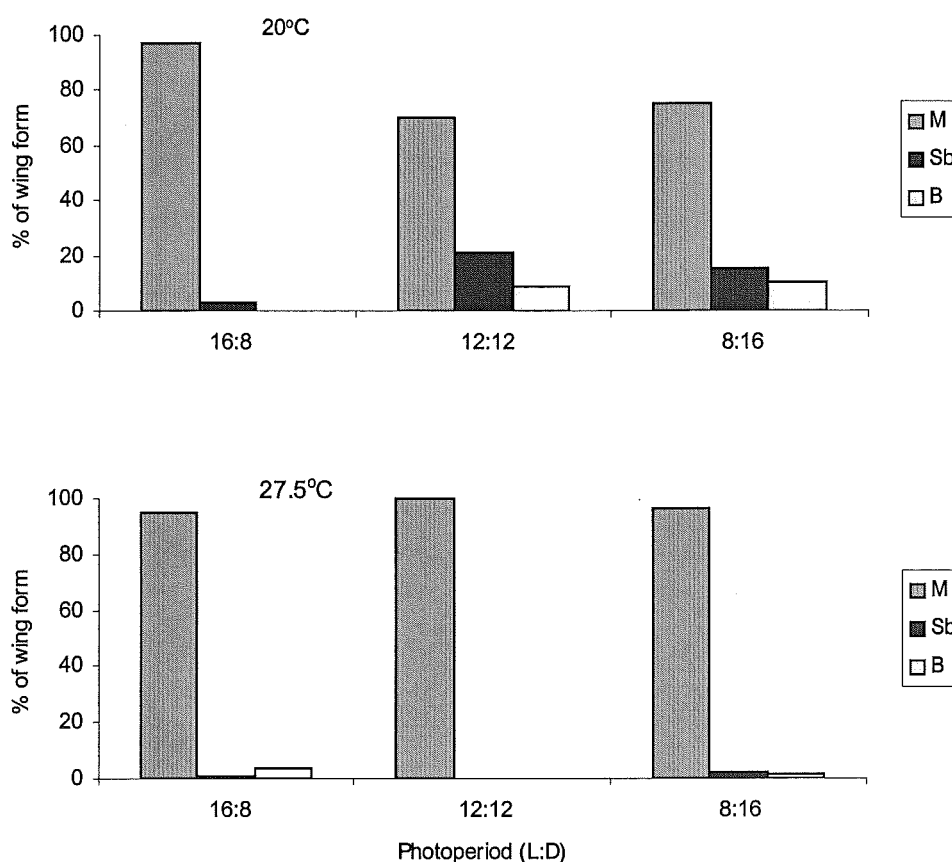


Fig. 7-4. Effect of photoperiod on wing form of *N. huttoni* in laboratory experiments. **M** - macropterous; **Sb** - sub-brachypterous; **B** - brachypterous.

Also, it can be seen that the differences in percentages of both the Sb- and B-forms between the 16-h (Sb-form 2.8% and B-form 0%) and 12-h photoperiods (Sb-form 21% and B-form 8.6%) were larger than those between the 12-h and 8-h photoperiods (15%, which was lower than that at the 12-h photoperiod, in

the Sb-form and 10% in the B-form). This may indicate that the effect of photoperiod on reduction in wing length becomes weaker as day length shortens below 12 hours.

At 27.5°C, the response of *N. huttoni* to photoperiod in terms of wing length was not as apparent as at 20°C (Table 7-6). The proportion of the M-form was very high at all three photoperiods tested, especially the proportions at 12-h (100%) and 8-h (96.7%). Under an 8-h photoperiod, 2.2% of the Sb-form and 1.1% of the B-form occurred, but these percentages were lower than those under the same photoperiods at 20°C. However, under the 16-h photoperiod, the B-form comprised 3.8% of individuals.

7.3.2 Flight

Temperature and flight. Table 7-7 shows the number and percentages of flying and nonflying adults collected from the field during the flight periods in 1997-1998 and 1998-1999. A total of 721 flying adults (341♀: 380♂ = 1: 1.11, $P > 0.05$, χ^2 test) were collected in 8 samples (5 in 1997-1998 and 3 in 1998-1999). When the flights occurred, air temperatures were 25-36°C and exceeded 30°C on 6 of the 8 sampling days. Recorded ground temperatures were 36-55°C, all being over 45°C except on 27 February 1998 (36°C). The ground temperature was on average 15°C (9-25°C) higher than air temperature on sampling days.

Air temperature and ground temperature fluctuated in the field, but multiple observations showed that as long as the air temperature rose to 30°C, and/or the ground temperature reached 40°C, flight took place, immediately. Furthermore, the higher the temperature, the more adults flew. Conversely, when the air temperature dropped to below 30°C, or ground temperature was below 40°C, no flights were observed. These two temperatures are therefore

Table 7- 7. Comparison of numbers and percentages of flying and nonflying *N. huttoni* in the field in 1997-1998 and 1998-1999.

Sampling date	Sampling time	Type of adult†	Sample size			Sex ratio (♀:♂)	Macropterous						Sub-brachypterous						Brachypterous						Temperature at time of sampling (°C)	
							No.			%			No.			%			No.			%				
			Total	♀	♂		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	Ground	Air
1997-1998																										
6/02/98	2:00-2:30pm	FI	80	39	41	1:1.05	39	41	80	48.8	51.3	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	55	36
7/02/98	2:00-2:30pm	FI	22	10	12	1:1.20	10	12	22	45.5	54.5	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	50	25
		Nf	200	95	105	1:1.11	95	105	200	47.5	52.5	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	48	32
17/02/98	12:30-1:30pm	FI	110	56	54	1:0.96	55	54	109	50.0	49.1	99.1	1	0	1	0.9	0.0	0.9	0	0	0	0.0	0.0	0.0	50	36
20/02/98	12:00-12:30pm	FI	51	26	25	1:0.96	26	25	51	51.0	49.0	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	45	30
		Nf	200	85	115	1:1.35*	84	107	191	42.0	53.5	95.5	1	4	5	0.5	2.0	2.5	4	0	4	2.0	0.0	2.0	39	26
27/02/98	12:30-1:30pm	FI	70	40	30	1:0.75	40	30	70	57.1	42.9	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	36	27
		Nf	200	83	117	1:1.41*	83	116	199	41.5	58.0	99.5	0	1	1	0.0	0.5	0.5	0	0	0	0.0	0.0	0.0	45	30
1998-1999																										
18/02/99	12:00-12:30pm	FI	180	80	100	1:1.25	80	100	180	44.4	55.6	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	49	33
		Nf	180	90	90	1:1.00	89	90	179	49.4	50.0	99.4	1	0	1	0.6	0.0	0.6	0	0	0	0.0	0.0	0.0		
		Nf	249	133	116	1:0.87	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
19/02/99	12:30pm	FI	138	62	76	1:1.23	62	76	138	44.9	55.1	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	45	31
		Nf	138	81	57	1:0.70*	81	57	138	58.7	41.3	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0		
		Nf	170	101	69	1:0.68**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
21/02/99	1:00pm	Nf	178	88	90	1:1.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	38	25	
22/02/99	1:00pm	Nf	114	60	54	1:0.90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	37	23	
25/02/99	1:00pm	Nf	120	55	65	1:1.18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33	22	
6/03/99	1:00pm	FI	70	28	42	1:1.50	28	42	70	40.0	60.0	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0	47	33
		Nf	70	33	37	1:1.20	33	37	70	47.1	52.9	100.0	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0		

[†]FI: Flying adults; Nf: Nonflying adults. *P < 0.05, **P < 0.01, X² test.

close to the thresholds for flight of *N. huttoni*. Most flights were observed between 12:00 noon and 3:00 pm.

Of the 721 flying adults taken in the 8 samples, none belonged to the B form and only one female was a Sb-form individual (Table 7-7). However, nonflying adults that were collected at the same time from the same population included Sb- and B-forms. For example, of the flying insects collected on 20 February 1998, the 51 adults (26 females and 25 males) were all M-form (100%), while in the ground population ($n = 200$), 2.5% were Sb-form and 2.0% were B-form. The Sb-form was also collected from the ground population on 27 February 1998 (0.5%) and 18 February 1999 (0.6%), but none were found flying. These findings suggest that the B-form might be flightless, and that flights of the Sb-form are rare.

A χ^2 test showed that the sex ratio of flying females to males (like the ground population) did not differ from 1:1 ($P > 0.05$), indicating that both sexes have the same temperature thresholds for flight. The sex ratio of flying adults in *Nysius vinitor* was also reported to be 1:1 (Kehat & Wyndham 1973a).

Age and flight. A total of 210 flying females and 200 nonflying ones from 5 samples were dissected to examine their developmental states, which were classified as: (1) immature (non-gravid), with no eggs formed in the ovarioles; (2) mature (gravid), with mature or developing eggs formed or forming in the ovarioles. Results (Table 7-8) show that the surface population comprised both mature (gravid) and immature (non-gravid) females when flights occurred, although the immature ones were predominant (70.0-96.8% of the female population). However, all flying females dissected were immature.

Starvation and flight. Flight activity was examined using 39 females and 33 males that were kept together to starve for 48 hours. Frequencies of flight from a tray over a period of 15 minutes were recorded and are given in Table 7-9.

In the process of testing, two interesting observations were made. First, repeated take-offs by some individuals in the tray were seen. These individuals landed in the tray immediately after take-off or they flew away from the tray. Second, some other individuals never took off during the entire test. This indicates there may be two types of individuals, "flyers" and "non-flyers".

Table 7- 8. Relationships between state of reproductive maturity and flight in field-collected *N. huttoni*.

Sampling date	Type of female adult*	No. female adults collected	Gravid females		Non-gravid females	
			No.	%	No.	%
27/02/98	Fl	40	0	0.0	40	100.0
25/02/98	Nf	30	9	30.0	21	70.0
18/02/99	Fl	80	0	0.0	80	100.0
	Nf	80	5	6.3	75	93.8
19/02/99	Fl	62	0	0.0	62	100.0
	Nf	62	2	3.2	60	96.8
6/03/99	Fl	28	0	0.0	28	100.0
	Nf	28	3	10.7	25	89.3

*Fl: Flying female adult; Nf: Nonflying female adult.

Table 7- 9. Relationships between starvation and flight activity of *N. huttoni* in the laboratory.

Sex	No. adults tested	Testing time (min.)	Frequency of take-off		Adults flown away from tray	
			No.	%	No.	%
Female	39	15	24	61.5	12	30.8
Male	33	15	15	45.5	4	12.1

The test was conducted in the laboratory on 29 February 1998. The temperature was about 25°C during test. A U-test was used to compare the percentage of take-offs and the percentage of male and female insects that flew away from the tray. $P > 0.05$.

The frequency of take-offs by the 39 females was 61.5% in 15 minutes; 12 females (30.8%) flew away from the tray. The frequency of take-offs by the 33 males was 45.5% and 4 (12.1%) flew away from the tray. The frequency of take-offs and the numbers of the individuals that flew away was higher in females than males, but not significantly different ($P > 0.05$, U-test).

Sexual deprivation and flight. A total of 100 females and 100 males collected from the field were kept separately from each other for two days (48 hours), and offered food. Males and females were then placed in separate trays to test their flight activity over 15 minutes (as above). Results are given in Table 7-10 and showed that the prior lack of mates greatly enhanced flight in both sexes. The frequency of take-offs was 155% in females and 161% in males during 15 minutes, but not one of either sex flew away from the tray before the test ended.

It is assumed that individuals took off to seek a mate. One adult that took off tried to mate with another one immediately on landing. When it recognised that it was of the same sex, it released that individual. It then took off and landed and tried to mate several more times. Such behaviour was observed for both sexes.

Table 7-10. Relationships between sexual deprivation and flight activity of *N. huttoni* in the laboratory.

Sex	No. adults tested	Testing time (min.)	Frequency of take-off		Adults flown away from tray	
			No.	%	No.	%
Female	100	15	155	155.0	0	0.0
Male	100	15	161	161.0	0	0.0

The test was conducted in the laboratory on 5 March 1998. The temperature was about 25°C during test.

Light and flight. Twenty females and 20 males collected on 31 March 1998 were kept together for 24 hours with the opportunity to mate and feed. Their flight test was conducted for 15 minutes at 25°C in the laboratory on 1 April 1998. Frequency of take-offs of 40 sexually mixed adults was 35%, but none flew away from the tray. They were then taken outside and tested again in sunlight at 2:00 pm on the same day. The air temperature was 30°C and ground temperature was 32°C during the test. Frequency of take-offs was 287.5% in 15 minutes and 12 (30%) individuals flew away from the tray. The 28 individuals left were then moved into the shade where air and ground temperatures were both 25°C and tested again for 15 minutes. No take-offs occurred in the shade over this period. When the insects were moved into sunlight again, take-offs occurred immediately and 77 (275%) were recorded in 15 minutes. Ten (35.7%) individuals flew away from the tray. Eighteen *N. huttoni* (13 females and 5 males) were left when the test ended, indicating that slightly more males flew away than females. These results indicate that the flight of *N. huttoni* is influenced by light.

What should be pointed out is that the effect of light on flight is associated with temperature. Usually, the shade is cooler than sunlight.

7.4 Discussion

7.4.1 Wing polymorphism

Proportions of wing forms in natural populations. Proportions of the three wing forms of *N. huttoni* adults differed greatly in field populations. The M-form predominated, forming about 94% of the population when the three years of data were summed, whereas the Sb-form comprised 5.5%, and the B-form did not exceed 1% of the population.

In the natural population, the M-form was more likely to develop in males than in females, while the proportion of the Sb-form was higher in females than males ($P < 0.01$, χ^2 test). Conversely, Fujisaki (1989b) found a higher proportion of macropterous females than males of the oriental chinch bug, *Cavelerius saccharivorus* Okajima. The ecological significance of these differences is unknown.

Johnson (1969) pointed out that migratory behaviour has often evolved in species whose habitats periodically become adverse for breeding, or disappear altogether. Fully developed wings (the M-form) allow the possibility of escape from degraded or overpopulated habitats, and increase the possibility of colonisation of new sites. The possession of wings enables seasonal changes in environmental conditions to be countered. The M-form can also make better use of temporal or ecologically more diversified habitats than the Sb- and B-forms.

As pointed out by Honěk (1976a), wing forms are controlled by two kinds of determinants: intrinsic (hereditary factors) and extrinsic (environmental factors). The main environmental factors influencing wing form are crowding, temperature, photoperiod, food, and occasionally also other factors. One factor is usually the principal, and others modify its action.

Effect of temperature and photoperiod on wing forms. The present study indicates that both low and high temperatures and short day-length at lower temperature tend to accelerate the production of the Sb- and B-forms. In the field the rise in frequencies of the Sb-form towards autumn (1995-1996) exemplify this.

Fujisaki (1989b) reported that in the oriental chinch bug, *Cavelerius saccharivorus* Okajima, high temperature (30°C) and long photoperiod (16L:8D) in the nymphal stage stimulated the appearance of macropters.

Conversely, low temperature (20°C) and short photoperiod (8L:16D) favoured the appearance of brachypters. A similar report was given for *Pyrrhocoris apterus* by Honěk (1976a) who found that an 18-h long-day photoperiod stimulated the development of macropters, while a 12-h photoperiod inhibited it. Photoperiodic reaction was affected by temperature. Under long-day conditions, the maximal proportion of macropters of *P. apterus* was produced at 25-27°C, whereas lower (21°C) as well as higher (31-33°C) temperatures decreased their proportion. In *Limnopus canaliculatus* (Say), reduction in photoperiod had a strong positive effect on the production of the long-winged overwintering morph (Zera & Tiebel 1991). Harade & Taneda (1989) who worked with a water strider, *Gerris paludum insularius* (Motschulsky) found that a 14.5-h long-day photoperiod during the whole nymphal stage caused more adults to be brachypterous than 12-h or 9.5-h photoperiods each day. Only under the 12-h photoperiod, was a significant effect of temperature observed on *G. paludum insularius*: high temperature (30°C) induced a higher proportion of macropterous adults than low temperature (20°C). Temperature and photoperiod have been reported to be responsible for the determination of wing forms in many other insect species, too, including *Chaetosiphon fragaefolii* (Schaefers & Judge 1971), *Acyrtosiphon kondoi* (Kodet & Nielson 1980), *Gryllus campestris* (McFarlane 1964), and *Pteronemobius taprobanensis* Walker (Tanake *et al.* 1976). Photoperiod, however, did not affect wing form in *Jadera aeola*, which produced only longed-winged adults at all photoperiods tested. Shorted-wing forms of *J. aeola* occurred in the field (Tanaka & Wolda 1987).

It is essential to consider environmental control of wing-form determination in the context of the life cycle of *N. huttoni*, which has been shown to have three generations each year. The first and second generations as well as a partial third generation occur mainly between spring and summer when high temperature and long day length predominate. Under high temperature/long photoperiod conditions, macropters are more adaptive than sub-brachypters

and brachypters because they can adjust their body temperatures by flight, or migrate to more favourable habitats when the weather is hotter than they can bear, or if it results in drying up of host plants. Therefore, the production of macropters in response to high temperature and long photoperiod is highly adaptive. More sub-brachypters are likely to appear in the third generation when temperatures became lower in autumn and a shortening photoperiod prevails. This is also adaptive because lower temperatures do not bring about flight, as discussed later in this Chapter.

I was unable to determine any decisive factor influencing wing length, which may be under genetic control in combination with multiple environmental factors (e. g., temperature, photoperiod, population density, and food).

Number of instars and B-form. In the course of rearing *N. huttoni* (Chapter 3), production of the B-form was found to be related to a reduction in the number of instars. Although the B-form included both 4- and 5-instar adults, it made up a higher proportion of 4-instars than 5-instars (Tables 7-11 and 7-12). For example, the B-form made up 50.0, 71.4, and 83.3% of each 4-instar adult population at 15, 30, and 35°C, respectively, while percentages of the B-form with 5 instars were 52.9% at 15°C, 9.3% at 20°C, and 7.2% at 35°C (Table 7-11). Similarly, at 27.5°C under 16-h photoperiod (Table 7-12), 3 of 4 B-forms were 4-instar adults, while only one was a 5-instar adult.

Within the B-form group with 4 instars, 2 males out of 3 reared under 16-h photoperiod at 27.5°C, 2 males out of 5 at 30°C, and 4 (3 females and 1 male) out of 5 at 35°C showed abnormal changes in wing development. As shown in Fig. 7-5 (A: ♀, B: ♂), the wings of these individuals were extremely short and lobe-like. Although the abnormal B-form was found only under laboratory conditions, its occurrence demonstrates that high temperature can interfere with wing development.

Table 7-11. Numbers and percentages of brachypterous-form adults produced in the laboratory at 6 experimental temperatures.

Temperature (°C)	Total no. B-form produced	4-instar adult			5-instar adult		
		No. adults	No. B-form	% of B-form	No. adults	No. B-form	% of B-form
15	10	2	1	50.0	17	9	52.9
20	7	5	0	0.0	75	7	9.3
25*	0	0	0	----	47	0	0.0
30	5	7	5	71.4	102	0	0.0
35**	21	6	5	83.3	195	14	7.2
L-T*	0	0	0	----	120	0	0.0

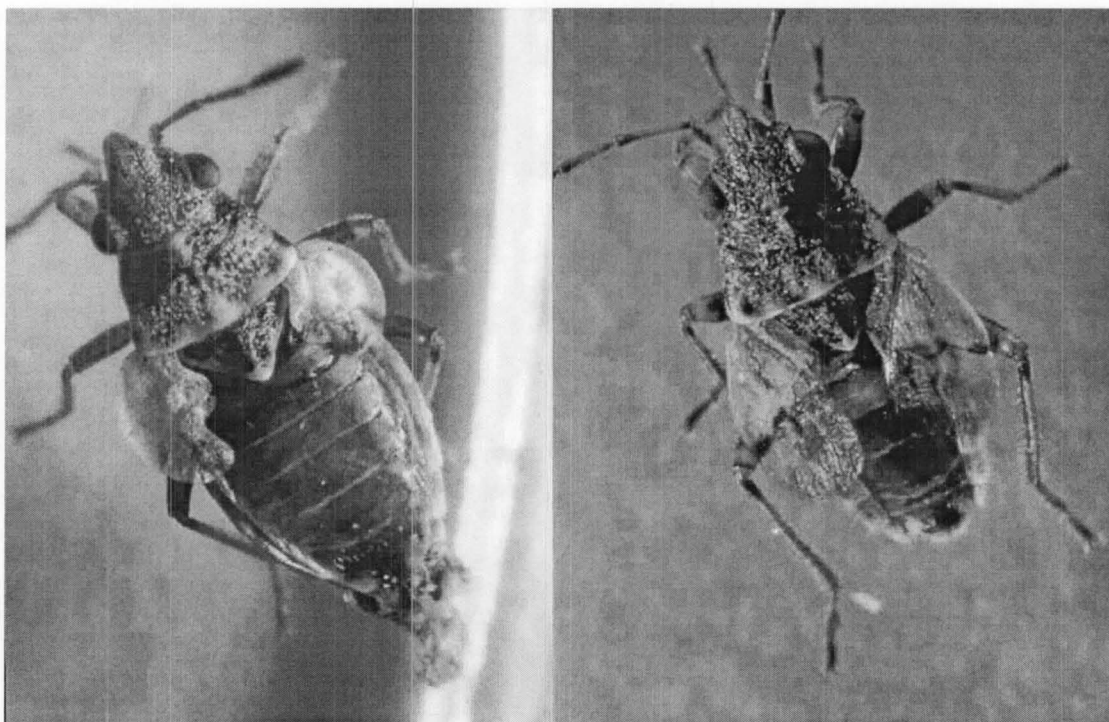
*No B-form and 4-instar adults occurred at 25°C and lab temperature (L-T). **2 B-form adults at 35°C were 2 individuals out of eleven 6-instar adults (18.2%).

Table 7-12. Numbers and percentages of brachypterous-form adults produced in the laboratory at 2 temperatures and 3 photoperiods.

Temperature (°C)	Photoperiod (L:D)	Total no. B-form produced	4-instar adult			5-instar adult		
			No. adults	No. B-form	% of B-form	No. adults	No. B-form	% of B-form
20	16:8*	0	0	0	----	71	0	0.0
	12:12	7	5	0	0.0	75	7	9.3
	8:16	4	3	0	0.0	36	4	11.1
27.5	16:8	4	7	3	42.9	97	1	1.0
	12:12**	0	4	0	0.0	106	0	0.0
	8:16	1	10	0	0.0	78	1	1.3

*No B-form and 4-instar adults occurred at 16-h photoperiod at 20°C. **No 4-instar adults occurred at 12-h photoperiod at 27.5°C.

Wing polymorphism is influenced by the amount of juvenile hormone and moulting hormone in insects. Southwood (1961) considered the brachypterous forms of Heteroptera were either adults with juvenile characters (metathetely) caused by an excess amount of juvenile hormone (JH), or larval forms with adult characters (prothetely) caused by a lessening effect of JH. He suggested that distinguishing between metathetely and prothetely might be done by considering the number of instars passed through; thus, when a morph with



A: ♀

B: ♂

Fig. 7-5. Brachypterous *N. huttoni* with very short wings produced at 35°C. Both individuals show had 4 nymphal instars.

some juvenile characters is produced after the normal number of instars, the condition may be considered to be metathetely. If, however, there is a reduction in the number of instars, then the morph produced is an example of prothetely. The former results from a lengthening of the period of influence of the juvenile hormone and the latter from a reduction in this period. The former is also associated with cold conditions and the latter with hot conditions (Southwood 1961).

Wigglesworth (1952) found that hormone balance in *Rhodnius prolixus* was upset by abnormal temperature, and he found that the 4-instar nymph would not moult at 36°C. High temperature was shown to depress the action of

juvenile hormone in *Rhodnius* so the insect shows a mild degree of prothetely. In contrast, low temperature slightly enhances the action of juvenile hormone so that a mild degree of metathetely results. Wigglesworth also found that low temperature upsets the hormone balance very slightly in favour of juvenile hormone, while high temperature upsets the balance slightly in favour of the moulting hormone.

The findings of Southwood and Wigglesworth indicate that the B-form of *N. huttoni* produced at 15°C is an example of metathetely. Conversely, the B-forms, especially those with 4 instars, produced at 30 and 35°C may be regarded as examples of prothetely. Presumably, the increase in proportion of the B-form at both lower and higher temperatures is associated with changes in hormone balance at lower ($\leq 15^{\circ}\text{C}$) and higher ($\geq 28^{\circ}\text{C}$) temperatures.

Genetics. In many cases, wing polymorphism and associated flight ability are more or less genetically determined (Solbreck 1986). Solbreck studied wing and flight muscle polymorphism in a lygaeid bug, *Horvathiolus gibbicollis* and found that all F_1 offspring between crosses of macropterous and brachypterous forms were brachypterous. In the F_2 generation, however, a 3:1 ratio of brachypters to macropters appeared, suggesting that the brachypter is determined by a single dominant gene. Zera *et al.* (1983) also studied wing polymorphism in the waterstrider *Limnoporus canaliculatus* using crossing experiments between forms. They found that the proportion of macropterous progeny produced by the M x M cross was greater than the proportions produced by any other crosses. In *Pyrrhocoris apterus* (Honěk 1976a), the proportion of macropters was raised by selection to 80% in a laboratory strain, whereas a nonselected strain comprised only about 2% of macropters at the same time, indicating that wing polymorphism was also genetically determined.

The genetic basis of wing-form determination in *N. huttoni* is not yet understood. In my study of mating combinations among three wing forms (Table 7-3), all possible mating combinations were not found in natural populations, but the B-form was involved in 5 mating combinations. These were ♀M x ♂B and ♂M x ♀B in M x B, ♀Sb x ♂B and ♂Sb x ♀B in Sb x B, and B x B. Due to very low proportions of the B-form in both sexes (about 0.4%), the proportions of these 5 mating combinations are either very low (♂M x ♀B: 1.1% and ♂Sb x ♀B: 0.1% only in 1996-1997) or do not occur (♀M x ♂B, ♀Sb x ♂B, and B x B). The occurrence of ♂M x ♀B was the result of an increase in the proportion of the B-form in the field in spring and early summer. This increased the opportunity for copulation between ♂M and ♀B individuals.

Conversely, the proportion of the mating combination M x M was the highest in the field (about 81%, Fig. 7-2); and was a consequence of very high proportions of M-form individuals (92.8% of females and 95.2% of males) in the field.

My results revealed the number of mating combinations among three wing forms, and the percentage of each combination of *N. huttoni* in a natural population for the first time. Detailed experiments using different cross combinations among wing forms will be necessary to obtain a more detailed understanding of genetic control of wing forms in this species.

Crowding. It has been reported that crowding of nymphs is one of the factors influencing wing length in hemipterans and usually stimulates the appearance of macropters. For example, Fujisaki (1989b) found that in the oriental chinch bug, *Cavelerius saccharivorus* Okajima, crowded rearing stimulated the production of more macropters, while isolated rearing resulted in more brachypters. He also pointed out that crowding exerted such an effect only in conditions of high temperature and/or long photoperiod. Braune (1983) who worked with female *Leptoptera dolobrata* found that in field surveys, the

highest percentages of macropterous females were always found in habitats where *L. dolobrata* occurred at relatively high densities. To test whether this was a consequence of crowding, Braune reared field-collected first instar nymphs in the laboratory at densities of 25, 50, 100, 200, and 400 individuals per cage until the adults emerged. His results showed that a higher percentage of experimentally crowded nymphs developed into macropterous females than individuals reared at lower densities. Crowding was also the main factor responsible for the production of alatae in the aphid, *Chaetosiphon fragaefolii* (Judge & Schaefers 1971), and for the development of macropters in the brown planthopper, *Nilaparvata lugens* Stål (Kisimoto 1956), and the salt marsh-inhabiting planthopper, *Prokelisia marginata* (Denno 1979).

As pointed out by Honěk (1976b), it is necessary from an ecological point of view to evaluate whether the varying proportions of wing forms found in natural populations are consistent with experimental results obtained under laboratory conditions. My surveys showed that the M-form of *N. huttoni* was the predominant form in the field where it made up about 94.1% of the study population. This value was higher than the 64.0-78.7% of M-forms obtained by rearing at various temperatures in the laboratory, and the differences may have been due to crowding. However, all nymphs were reared separately (one vial contained one nymph) in my studies until the adults emerged, so crowding could not have affected wing length in the laboratory. Clearly, it will be necessary to carry out experiments to confirm whether or not crowding is one of the factors affecting wing length in *N. huttoni*.

7.4.2 Flight

Temperature and flight. My field observations showed that temperature was a major factor associated with flight of *N. huttoni*. Thus, when temperature reaches a threshold value, flight occurs, and then increases with increasing temperature. For example, between 2:00 – 2:30 pm on 6 February 1998,

ground and air temperatures were 55°C and 36°C, respectively. At that time, a large number of adults were flying and 80 adults were collected in half an hour. On the next day, however, the number of adults seen flying was low presumably due to the lower air temperature (25°C) although the ground temperature was still very high (50°C). Thus, only 22 adults were collected in half an hour. This suggests that the flight of *N. huttoni* is caused or influenced by high temperature (over 30°C) and that both sexes are sensitive to high temperature. Similarly, Zhang & Shipp (1998) reported that flight activity of *Orius insidiosus* increased with increased temperature. However, Dingle (1968) found that both high (27°C) and low (19°C) temperatures seem to depress flight in *Oncopeltus fasciatus* (Dallas).

Most Hemiptera are predominantly day fliers (Southwood 1960; Lewis & Taylor 1965), a condition that is thought to reflect their high flight temperature thresholds. In the present study, flights of *N. huttoni* occurred during the middle of the day (12:00-3:00 pm), and none were observed at the beginning or end of the day or at night when temperatures are lower. These observations are consistent with findings for *Oncopeltus fasciatus* (Dallas) (Dingle 1968), however, *Nysius vinitor* (Kehat & Wyndham 1973a) was reported to fly during both day and night, the night peak occurring in late evening (9:00-9:30 pm).

The distance of flights and the height of take-offs in *N. huttoni* were not very great and ranged from less than a meter to about 5 meters or a little longer. Many hop-like flights occurred near the ground and appeared to be food searching activities stimulated by high temperature. Longer flights that were observed when the vegetation had dried up may be classed as migratory flights, although there appeared to be no sharp line of demarcation between the two types of flight.

Age and flight. Migration of females in a wide range of species often occurs when the adult is sexually immature (Johnson 1960). As noted in Chapter 4,

generations of *N. huttoni* overlap in the field, and new adults emerge continually as a consequence of on-going egg-laying by females. Most of the time, therefore, females in the field are a mixture of egg-laying (gravid) and immature (non-gravid) insects. The fact that all flying adult females were immature indicates a fundamental difference in flight behaviour between immature and mature females. In *Nysius vinitor*, most females migrate when immature although a small proportion of them disperse as egg-laying adults (Kehat & Wyndham 1973a). Similarly, Waloff & Bakker (1963) found that in five species of Miridae, *Heterocordylus tibialis* (Hahn), *Asciodema obsoletum* (fieber), *Orthotylus adenocarpi* (Perris), *O. virescens* (Douglas and Scott) and *O. concolor* (Kirschbaum), some mature individuals could fly and disperse, but most flying females were immature. Only 12% of dissected *Heterocordylus* were mature, 7% of *Asciodema*, 11% of *Orthotylus adenocarpi*, 15% of *O. virescens* and none of the *O. concolor* females. These data are consistent, at least partially, with the results obtained for *N. huttoni*.

Laboratory rearing showed that *N. huttoni* males were sexually mature upon emergence, and that newly-emerged males could mate with females on the day they emerged. All males caught in flight or on the ground were sexually mature. Their flight behaviour in relation to sexual maturity therefore differs from that of females.

During ontogenesis, the ovaries and flight apparatus develop differentially, and the development of either system can be influenced by environmental factors. As shown repeatedly in migrating species, the adult life of females is characterised by an "oogenesis-flight syndrome" (Johnson 1969) such that the development of the flight apparatus retards maturation of the ovaries and dispersal flights occur before the onset of reproduction.

If migrations of females to new habitats occur, as noted above, the optimum time for migratory flight would be the pre-reproductive period, for mechanical

reasons related to wing-loading. On the basis of body organ mass, it can be deduced that the body weight of sexually immature (non-gravid) females does not differ drastically from that of males and the two groups should have similar degrees of wing-loading. However, the body weight of mature (gravid) females increases enormously as ovarian development progresses. This increase in weight results in a higher degree of wing-loading, which consequently increases the power requirements for flight. Based on this criterion, the occurrence of dispersal flights during the pre-reproductive phase of adult life, as seen in *N. huttoni*, would seem to be a distinct advantage. Therefore, the phenomenon that mature females in the field apparently do not fly is fully consistent with the "oogenesis-flight syndrome" (Johnson 1969).

Starvation and flight. The results of my tests on the relationship between starvation and flight suggest that flight in *N. huttoni* can also be a response to food shortage in the habitat, and that adults fly in search of food. This is a subjective interpretation based on my observation that over 95% of the vegetation, whether food plants or not, was dried up when flight occurred. Thus, collections of flying adults were made on 5 sampling days in 1997-1998 and 2 sampling days in 1998-1999 when vegetation was largely dried up, but not on 6 March 1999 when the vegetation was recovering.

Dingle (1968) studied the effect of starvation on flight of *Oncopeltus fasciatus* (Dallas), which were 5-8 days and 20-28 days old and had been deprived of food but not water for four days. He concluded that food deprivation increased the proportion of emigrants no matter whether starvation occurred shortly after eclosion or after oviposition had begun. In studies of three other species, *Dysdercus fasciatus* Sign, *D. nigrofasciatus* Stål, and *D. superstitiosus* (F.), Dingle & Arora (1973) found that starved females of all three species undertook longer flights than fed ones. After studying the relationship between food, age, and flight in *Nysius vinitor*, Kehat & Wyndham (1973b) reported that inadequate food consumption during either the nymphal, adult, or both stages

may increase the likelihood of flight in adults. Also, they reported that starvation of both immature and mature adults resulted in an increase in both take-offs and flight duration. Similarly, Solbreck & Pehrson (1979) found when the seed bug, *Neacoryphus bicrucis* (Say) was starved, flight was much enhanced, whereas fed individuals exhibited very little flight. In my experiments, adults used in tests were collected from the field. Although their ages were not known, deprivation of food for two days was followed by take-offs by 45.5% of males and 61.5% of females. Furthermore, 12.1% of males and 30.8% of females flew away from the testing tray. These results suggest that flight activity brought about by starvation is adaptive as it may allow short-range displacement of much of the population into habitats where resources may be greater.

Flying adults were collected during two years and were all members of the second (1998-1999) and third generations (1997-1998). No flying adults of the first generation were collected, although flights of first-generation adults were observed in the field. First-generation adults occur mainly between mid-November and late January (Fig. 4-1) when high temperatures sometimes bring about flights of adults. For example, in December 1996 nearly all vegetation in habitats of *N. huttoni* had dried up and the adult population was very small. At such times, adults appear to "look for" new environments due to the shortage of food. *N. huttoni*, therefore, may show two types of flying activity, as recorded for broom mirids (Waloff & Bakker 1963): (a) movements associated with short-term high temperatures, or take-offs inside their habitat, and (b) movements to new habitats associated with shortage of food due to drying up of vegetation. Because high temperatures usually do not last long (generally a few days only) take-offs of insects under hot conditions probably enable short-term adjustments of body temperature, through shade seeking.

Sexual deprivation and flight. Responses to mate shortage and starvation by *N. huttoni* were not identical. Flight was instigated or enhanced by both

conditions, but the response of bugs to mate shortage was stronger than that to food shortage. Thus, frequency of take-offs was much higher in both females and males when they were separated from each other for some time than when they were starved. However, no adults flew away from the testing tray in response to mate shortage, whereas 12.1% of males and 30.8% of females did so in response to starvation. This may indicate that mate shortage induces shorter flights than food shortage as found in another lygaeid species, *Neacoryphus bicrucis* (Say) (Solbreck & Pehrson 1979).

Note however, that my study was done in the laboratory and separation of males from females may be uncommon in the field. Although the proportions of males and females differed significantly in some seasons, it is unlikely to be so great that it diminishes the chance of copulation and results in flight. Thus, there was no significant difference in the proportions of males and females in all collections of flying adult, and no flight was observed in the field on most sampling days (an exception was 4 February 1996) when males were present in significantly higher numbers than females.

CHAPTER EIGHT

Reproductive diapause in *N. huttoni*

Chapter 8

Reproductive diapause in *N. huttoni*.

8.1 Introduction

The climate and environmental conditions on the earth are changeable in space and time and these changes are either favourable or unfavourable for growth, development, and reproduction of insects (Zaslavaki 1988). Many insects living in highly variable environments have developed strategies such as migration and diapause to adapt to these changes (Behrens 1985). Migration permits escape in space and diapause permits escape in time (Solbreck 1978). Diapause is always initiated by environmental stimuli long before the unfavourable conditions set in (Behrens 1985). Diapause is an important adaptive mechanism for insects to cope with environmental changes (Beck 1968).

Depending on the species, diapause can occur in any of the developmental stages of insects: embryo, larva, pupa, or adult. Within the same species, however, diapause occurs at a specific stage and during a specific season (Behrens 1985, Tauber *et al.* 1986, Zaslavaki 1988). In some species with life cycles of a year or longer, diapause occurs in two or more stages. For example, the cockroach *Ectobius lapponicus*, which has a two-year life cycle, overwinters in the first year as a diapausing egg and in the second year as diapausing larva (Tauber *et al.* 1968). Diapause in the adult stage is called reproductive diapause (Beck 1968). The apparent character of adult diapause is that reproductive activity, particularly of egg maturation and oviposition, is arrested by certain environmental conditions (Beck 1968, Tauber *et al.* 1986).

Diapause is a genetically determined pattern of response to environmental stimuli. Among environmental stimuli, photoperiod is the most important factor in diapause induction, maintenance, and termination. Besides photoperiod, the

temperature is the second most important factor affecting diapause. Temperature can be influential in a variety of ways throughout course of diapause (Beck 1968, Lumme 1978, Hodek 1983, Behrens 1985, and Tauber *et al.* 1986). However, other environmental factors such as food, humidity, population density have also been known to influence insect diapause (Behrens 1985, Tauber *et al.* 1986).

N. huttoni goes through three generations a year in Christchurch, and overwinters as an adult (Fig. 4-1, Chapter 4). Adults of the third-generation that appear from early February to mid-May rarely oviposit prior to winter even though conditions for egg laying might be favourable. Farrell & Stufkens (1993) found there were two generations of *N. huttoni* in Christchurch and that reproductive diapause occurred in the second generation. The present experiments were conducted to determine whether or not *N. huttoni* females enter reproductive diapause in later summer and early autumn and, if so, to determine the factors inducing and terminating diapause. Reproductive diapause in adult insects is manifested as a suppression of reproductive function (Beck 1968).

8.2 Materials and methods

8.2.1 Field observations on the natural induction of diapause.

These were conducted as part of the study of field biology (Chapter 4). After the adults of the third generation emerged, observations were made to determine whether or not nymphs of the new (4th) generation hatched from eggs in the field.

8.2.2 Examination of developmental state of female adults by dissection.

The method used has been described in 4.2.4 of Chapter 4 (page 80).

8.2.3 Artificial breaking of reproductive diapause by high temperature and long daylight.

Adults of the third generation collected from the field between 15-25 March 1997 were known to be in reproductive diapause as shown by their lack of eggs. Further adults collected on 28 March 1997 were reared in pairs, using the method described in Chapter 3. A constant temperature of 25°C and a photoperiod of 16L:8D were used to determine whether high temperature and long photoperiod would terminate reproductive diapause. The number of eggs laid per female was recorded daily. The experiment ended on 20 May 1997 when all females were ovipositing, regularly.

8.2.4 Effect of temperature and photoperiod on reproductive diapause

Adults for these experiments were collected from hibernation sites in autumn. They were kept in dark-coloured ice-cream boxes at 15°C and supplied with shepherd's purse as food.

Experiment 1. – termination, induction, and retermination of reproductive diapause. Two controls and one treatment were used in this experiment. In the treatment, the insects were exposed to three temperature/light combinations in sequence, the first one at 25°C/16L:8D, the second one at 20°C/8L:16D, and the third one again at 25°C/16L:8D. In the first stage, the insects were kept until all females started to lay eggs, i.e., diapause had ended. Then they were transferred to the second stage, in which all females entered diapause, at which time they were transferred to the third stage. The experiment ended when diapause terminated again. The two “controls” were 25°C/12L:12D and 25°C/8L:16D at which the insects were kept until the experiments ended. Twenty pairs of adults were tested in each control and treatment.

Experiment 2. – induction, termination, and reinduction of reproductive diapause. Two controls and one treatment were established as in Experiment 1. The two controls were 20°C/16L:8D and 20°C/12L:12D, whereas, the treatment had three stages: the first one at 20°C/8L:16D, the second one at 25°C/16L:8D, and the third one at 20°C/8L:16D again. In the first stage, the insects were kept until all of them entered diapause. They were then transferred to the second stage. After they started to lay eggs, the insects were transferred to 20°C/8L:16D again. The experiment ended when the females re-entered diapause. Twenty pairs of adults were included in each control and treatment.

8.3 Results

8.3.1 Field observations on the natural induction of diapause.

As shown in Fig. 4-1, adults of the third generation emerged between early February and mid-March. My field observations showed that no nymphs of the fourth generation appeared before winter although the weather was apparently favourable for laying eggs. This indicated that third-generation adults entered reproductive diapause in autumn.

8.3.2 Examination of developmental state of female adults by dissection.

Results of dissections of females are given in Table 4-3. The proportion of females that oviposited in the field apparently decreased starting in February. The proportion of third-generation adults that entered reproductive diapause increased as the season progressed. By 5 April, all females examined had entered diapause in both 1998 and 1999.

8.3.3 Artificial breaking of reproductive diapause by high temperature and long daylight.

Table 8-1 shows that all 10 females oviposited on average for 14 (10-21) days when transferred from the field to a long photoperiod of 16L:8D and a high temperature of 25°C. This indicated that the females were capable of terminating naturally-induced diapause when exposed to high temperature and a long photophase. Over the 53 days of the experiment, the mean oviposition period was 30.4 (7-42) days during which the total mean number of the eggs laid per female was 160.5 (18-266), a mean of 5.3 (2.6-7.2) eggs per female per day.

Table 8-1. Oviposition activity of *N. huttoni* when reproductive diapause was broken in the laboratory at 25°C and 16L:8D.

	Pre-oviposition period from collection to oviposition (in days)	No. eggs laid per female during experiment	Oviposition period during experiment (in days)	Daily mean eggs laid per female.
Average	13.9	160.5	30.4	5.3
Range	10 - 21	18 - 266	7 - 42	2.6 - 7.2

Ten females that were collected from the 3rd generation population in the field were used. The experiment was conducted between 28 March and 20 May 1997. Three females died before the experiment ended.

8.3.4 Effect of temperature and photoperiod on reproductive diapause

Experiment 1. – termination, induction, and retermination of reproductive diapause. Fig. 8 shows the effect of temperature and photoperiod on reproductive diapause. Results obtained from the treatment in Experiment 1 are given in Table 8-2. In the first stage, which lasted for 11 days, 19 (95%) out of 20 females oviposited after transfer to high temperature (25°C) and long photoperiod (16L:8D). The mean pre-oviposition period was 4.6 (4-7) days. This confirmed that reproductive diapause terminates at high temperature and long photoperiod, as shown in Table 8-1. Four females died and 16 were transferred to the second stage at 20°C/8L:16D.

Treatment	1st stage (11 days)	2nd stage (61 days)	3rd stage (30 days)
	25°C/16L:8D	20°C/8L:16D	25°C/16L:8D
	95% (19) females terminated diapause within average 4.6 days.	100% (9) females entered diapause within average 30 days.	100% (2) females reterminated diapause within average 6.5 days.
Control 1	25°C/12L:12D (102 days)		
	90% (18) females terminated diapause within average 5.1 days.		
Control 2	25°C/8L:16D (102 days)		
	90% (18) females terminated diapause within average 11.8 days.		

Experiment 1: Termination, induction and retermination of reproductive diapause of *N. huttoni*.

Treatment	1st stage (63 days)	2nd stage (12 days)	3rd stage (80 days)
	20°C/8L:16D	25°C/16L:8D	20°C/8L:16D
	80% (16) females oviposited with mean 8.4 days; and then 100% (13) females entered diapause within average 32 days.	100% (9) females terminated diapause within average 6.6 days.	100% (7) females re-entered diapause within average 24 days.
Control 1	20°C/16L:8D (155 days)		
	85% (17) females terminated diapause within average 7.5 days.		
Control 2	20°C/12L:12D (155 days)		
	95% (19) females terminated diapause within average 12.1 days.		

Experiment 2: Induction, termination and reinduction of reproductive diapause of *N. huttoni*.

Fig. 8. Summary of the effects of temperature and photoperiod on reproductive diapause in *N. huttoni*.

Insects were kept in the second stage for 61 days, during which 14 females continued to oviposit. However, 11 females died, 10 of them died before entering diapause. Nine females stopped ovipositing and entered diapause an average of 30 (4-47) days after transfer from the first stage. Five females were transferred to the third stage at 25°C/16L:8D, 22 (13-34) days after they entered diapause.

Table 8-2. Effect of temperature and photoperiod on termination, induction and retermination of reproductive diapause in *N. huttoni* in experiment 1.

1st stage: 25°C, 16L:8D (11 days)		2nd stage: 20°C, 8L:16D (61 days)								3rd stage: 25°C, 16L:8D (30 days)				No. adults alive when experiment ended	
	No. females that laid eggs	Pre-oviposition period from collection to oviposition (in days)	No. females transferred from previous stage	No. females that laid eggs	Oviposition period (no.days after which adults entered diapause) (in days)	No. adults died before entering diapause	No. adults that entered diapause	No. days after which insects were transferred to next stage	No. females transferred from previous stage	No. adults that terminated diapause	No. days after which diapause terminated	Oviposition period (in days)	No. adults died before diapause terminated	Female	Male
Mean	19	4.6	16	14	29.7	10	9	21.6	5	2	6.5	15.0	3	1	0
Range	---	4 - 7	---	---	4 - 47	---	---	13-34	---	---	4-9	4 - 26	---	---	---

Twenty female adults were tested. 4 died in the first stage and 11 died in the 2nd stage.

In the third stage, three females died within 2-9 days without ovipositing and the two others reterminated diapause and laid eggs within an average of 6.5 (4-9) days. When the experiment ended, only one female was still alive.

Results obtained for controls 1 and 2 in Experiment 1 are given in Table 8-4. At 25°C, the pre-oviposition period from collection to oviposition was not significantly longer under the 8:16 h photoperiod (11.8 days) than under the 12-h photoperiod (5.1 days) ($P > 0.05$, T-test), whereas the mean number of eggs laid daily by each female was marginally lower at 8:16 h (4.5 eggs/day) than at 12:12 h photoperiod (6.2 eggs/day) ($P = 0.05$, T-test).

Experiment 2. – induction, termination, and reinduction of reproductive diapause. Table 8-3 gives the results obtained from the treatment in Experiment 2. In the first stage at 20°C/8L:16D, 16 (80%) out of 20 females oviposited on an average of 8.4 (4-16) days. This high incidence of oviposition could be attributed to the exposure of insects to a higher temperature (20°C) than that (15°C) at which the insects were kept for experiments. Three females died before they entered diapause, and 13 entered diapause within 1-49 days. Eleven females were transferred to the second stage at 25°C/16L:8D, 5-31 days after they diapaused.

In the second stage, two females died before diapause ended, but the other 9 females terminated diapause and laid eggs an average of 6.6 (6-10) days later. They were transferred to the third stage at 20°C/8L:16D, 3-7 days after oviposition. In the third stage, 7 females stopped laying eggs and re-entered diapause within an average of 24 (3-73) days. The experiment ended an average of 12.5 (10-16) days after insects had re-entered diapause, when only one female was still alive.

Results from controls 1 and 2 kept at 20°C in Experiment 2 are also shown in Table 8-4. The period from collection to oviposition was not significantly longer

Table 8-3. Effect of temperature and photoperiod on induction, termination and reinduction of reproductive diapause in *N. huttoni* in experiment 2.

		1st stage: 20°C, 8L:16D (63 days)						2nd stage: 25°C, 16L:8D (12 days)					3rd stage: 20°C, 8L:16D (80 days)					No. adults alive when experiment ended	
	No. females that laid eggs	Pre-oviposition period from collection to oviposition (in days)	Oviposition period (no. days after which adults entered diapause) (in days)	No. adults died before entering diapause	No. adults that entered diapause	No. days after which insects were transferred to next stage	No. females transferred from previous stage	No. adults that terminated diapause	No. days after which diapause terminated	Oviposition period (in days)	No. adults died before diapause terminated	No. females transferred from previous stage	No. females that laid eggs	Oviposition period (no. days after which adults entered diapause) (in days)	No. adults died before entering diapause	No. adults that entered diapause	No. days between diapause and end of experiment	Female	Male
Mean	16	8.4	32.0	3	13	13.3	11	9	6.6	6.1	2	9	7	24.0	1	7	12.5	1	0
Range	—	4 - 16	1 - 49	—	—	5-31	—	—	6 -10	3 - 7	—	—	—	3 - 73	—	—	10-16	—	—

Twenty female adults were tested. 9 died in the first stage and 2 died in the 2nd stage.

Table 8-4. Effects of temperature and photoperiod on reproductive diapause of *N. huttoni*. (Results for controls in experiments 1 and 2).

Experimental conditions			No. females that laid eggs	Pre-oviposition period from collection to oviposition (in days)	No. eggs laid per female	Oviposition period (in days)	Daily mean eggs laid per female	No. adults alive when experiment ended	
								Female	Male
Experiment 1	25°C/12L:12D (control 1)	Mean	18	5.1	184.6	29.7	6.2	0	1
		Range	---	4 - 9	9 - 480	2 - 80	0.5 - 8.6	---	---
	25°C/8L:16D (control 2)	Mean	18	11.8	156.1	34.8	4.5	1	4
		Range	---	4 - 70	3 - 357	5 - 70	0.4 - 10.4	---	---
Experiment 2	20°C/16L:8D (control 1)	Mean	17	7.5	169.1	45.8	3.7	0	2
		Range	---	4 - 12	46 - 397	20 - 126	1.6 - 5.3	---	---
	20°C/12L:12D (control 2)	Mean	19	12.1	174.7	65.5	2.7	3	0
		Range	---	4 - 95	1 - 410	1 - 140	0.8 - 5.2	---	---

20 female adults were used in each control.

under a 12:12 h photoperiod (average 12.1 days) than under a 16:8 h photoperiod (7.5 days) ($P > 0.05$, T-test). However, the daily mean eggs per female was significantly lower at 12:12 h (2.7 eggs/day) than at 16: 8 h (3.7 eggs/day) ($P < 0.05$, T-test).

8.4 Discussion

Confirmation of reproductive diapause. Field observations, female dissections, and results of experiments on the artificial breaking of diapause indicate that adults of the third generation undergo reproductive diapause in late summer and autumn, and that this diapause is terminated in all individuals exposed to warm temperature (25°C) and long photoperiod (16L:8D). Similar results were found for *Orius insidiosus* by Kingsley & Harrington (1982), who collected females in the autumn and maintained them for 20 days at four photoperiod/temperature regimes (25°C/16L:8D; 25°C/12L:12D; 12°C/16L:8D; and 12°C/12L:12D). All females of *O. insidiosus* kept under long-day and warm temperatures (25°C/16L:8D) broke diapause and laid eggs, whereas none of the females maintained in autumn-simulated conditions (12°C/12L:12D) laid eggs.

Induction of diapause. When switched from long day (16 h light)/high temperature (25°C) conditions to short day (8 h light)/low temperature (20°C) conditions, females entered diapause within about one month. This switch simulated the change in the natural photoperiod and temperature in late summer and autumn, indicating that shortening days and falling temperature are factors inducing reproductive diapause. However, of the two, photoperiod is the only one that changes seasonally with mathematical precision, and is not affected by changes in weather and climate. Therefore, it is likely to be more important than temperature in inducing and maintaining diapause of *N. huttoni*. *N. huttoni* females also lived much longer under short days (8L:16D) than long days (16L:8D) at 20°C.

Maintenance and termination of diapause. When adults were switched from short day (8 h light)/low temperature (20°C) conditions to long day (16 h light)/high temperature (25°C) conditions, diapause of females was terminated within about one week. This switch simulated the change in the natural photoperiod and temperature in spring, and indicated that reproductive diapause of overwintered adults is terminated by increasing day length and temperature. The termination of diapause is therefore synchronised with the appearance of a favourable season for growth and development.

Dissections of field-collected females confirmed that under natural conditions, termination of reproductive diapause started at the beginning of September (Table 4-3), and by mid-September nearly all females had terminated diapause and started to lay eggs. These findings indicate that the field population of *N. huttoni* ends reproductive diapause within about two weeks in early spring. This contention was supported by the results of my experiment on artificial breaking of diapause in which reproductive diapause of field-collected bugs was broken in an average of two weeks. Although diapause of the field population was broken under natural conditions in the field, the diapause of field-collected bugs was broken by constant temperature and under a fixed photoperiod in the laboratory. Nevertheless, the experimental and field results were the same.

The effects of temperature and photoperiod. Photoperiod and temperature are considered to be the primary factors that control reproductive diapause. Experimental evidence available suggests that photoperiod is the principal factor and that temperature modifies its effect (Neal *et al.* 1992). As reported for *Jalysus spinosus* (Elsey 1974), the combination of long days and warm temperature hastened the termination of diapause in *N. huttoni*. At identical temperatures, the pre-oviposition period was longer under short photoperiod than long photoperiod although no significant difference was found statistically ($P > 0.05$, T-test). For example, at 25°C, the pre-oviposition period was 11.8

days under an 8-h day, whereas it was 5.1 days under a 12-h day ($P > 0.05$, T-test). At 20°C, the pre-oviposition period under a 12-h day was 12.1 days, which was longer than that (7.5 days) under a 16-h day ($P > 0.05$, T-test). Temperature, however, appeared to modify the photoperiodic induction of diapause. Thus, the pre-oviposition period of females was shorter at 25°C (5.1 days) than at 20°C (12.1 days). Van den Meiracker (1994) studied the effect of temperature on photoperiodic induction of diapause in *Orius insidiosus* at 10L:14D, and found that diapause occurred at 18, 21, and 25°C, but not at 30°C and that diapause was terminated rapidly after transfer to 25°C/16L:8D. This is partially consistent with my results, although no diapause of *N. huttoni* was induced at 25°C.

Diapause-inducing/terminating effects of photoperiodic changes have been reported for many species of insects. Nechols (1988) reported that under laboratory conditions, short day length (11L:13D) maintained, and long day length (16L:8D) terminated diapause in *Anasa tristis* females that were collected from the field. In *Riptortus clavatus* (Numata & Hidaka 1982), diapause was induced under a short-day photoperiod (10L:14D) at 25°C and terminated when adults were transferred to a long-day photoperiod (16L:8D) at the same temperature. *Orius insidiosus* (Ruberson *et al.* 1991) exhibited a typical long-day response, with nearly all females entering reproductive diapause when kept under a short photoperiod (10L:14D). In contrast, fewer insects initiated diapause under longer photoperiods (12L:12D, 13L:11D, 14L:10D, and 15L:9D).

For *N. huttoni*, the photoperiods inducing diapause were not identical with those terminating diapause. My field observations and results shown in Fig 4-1 indicate that adults of the third generation emerged initially between early February (1998) and mid-March (1996) during which the photophase in Christchurch was ca. 14-12.5 hours (14 h 2 min. on 10 February and 12 h 36 min. on 12 March 1998). However, in early spring, overwintered adults

terminated diapause and started to lay eggs from early to mid-September during which the photophase was ca. 11-12 hours (10 h 56 min. on 29 August and 11 h 54 min. on 18 September 1998). Apparently, the photophase for the induction of diapause is longer than that for the termination of diapause.

Physiological adaptations of insects to temperature and day-length are closely interrelated. Under natural conditions, it is not easy to separate and evaluate the role of each of these factors in affecting diapause, because in the course of days and seasons, they tend to fluctuate synchronously.

Estimation of the critical photoperiod. The critical photoperiod (defined as the photoperiod causing 50% of insects to enter diapause) has been studied in many species of Hemiptera. The critical photoperiod for induction of reproductive diapause at 24°C was reported to be between 14 and 13.5 h light per day for *Nabis americanoferus* and *N. roseipennis* (Yeargan & Barney 1996); between 14 and 13 h light for *Corythucha cydoniae* (Neal *et al.* 1992) and *Riptortus clavalus* Thunberg (Numata & Hidaka 1982); between 16 and 15 h light for *Deraeocoris brevis* and between 15 and 14 h light for *Anthocoris tomentosus* (Horton *et al.* 1998); between 14.5 and 14 h light at 27°C for *Anase tristis* (Nechols 1988); and between 13 and 12 h light for *Orius insidiosus* (Ruberson *et al.* 1991). However, although no specific experiment was conducted to determine the critical photoperiod of *N. huttoni*, the adults of the third generation emerge mainly in February and March, when the critical photoperiod is estimated to be between 14 and 12.5 h light. It may therefore be slightly shorter than in many other hemipterans.

Sensitive stage of diapause. The life history stage which is sensitive to photoperiod differs in different species. When *Corythucha cydoniae* was transferred from long (14L:10D) to short days (12L:12D) as teneral adults and 14-day-old adults, the percentage of females ovipositing in each group began to decline compared with the adults maintained under constant long days

(14L:10D) (Neal *et al.* 1992). The decrease was more abrupt for teneral females than for 14-day-old females. This indicated that the adult is sensitive to diapause-inducing photoperiods. When the bugs were transferred to short daylength as 5th instars and 4th instars, only 61% and 7% of females in each group began to oviposit. Oviposition ceased within three weeks, indicating that preimaginal stages also perceived and responded to diapause-inducing photoperiods. Furthermore, diapause was induced earlier in females that experienced short daylengths as 4th and 5th instar nymphs than in females that were transferred to short days as adults. In *Nabis americanoferus* and *N. roseipennis* (Yeargan & Barney 1996), the nymphal stage is most sensitive to diapause inducing photoperiods. In the present study, only adults of *N. huttoni* were used in experiments, so which stages respond to diapause-inducing conditions are not known in full. The combined field and laboratory data indicate that the adult is sensitive to photoperiod.

Intensity of diapause. The intensity of diapause, as determined by the length of time required for diapause termination, varies considerably among species. Furthermore, the intensity of diapause may also vary among individuals of the same species, depending on how long each has been exposed to diapause-inducing conditions. Diapause induced under different temperature/photoperiod conditions can also differ in intensity. For example, in *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae), diapause induced at 25°C was more intense than diapause induced at 20°C (Kalushkov *et al.* 2001) since females reared at 20°C showed significantly shorter pre-oviposition periods than those reared at 25°C. Similarly, in *Ostrinia nubilalis* (Lepidoptera: Pyralidae) temperatures of 25 and 22°C evoked more intense diapause than did 19°C, and within the diel range of scotophase (dark portion of the photoperiod), 12 h evoked more intense diapause than did either longer or shorter scotophases (Deck 1989). In my study, time required for breaking diapause was longer in naturally-induced bugs (14 days, Table 8-1) than laboratory-reared bugs (about 7 days both in the third stage in Table 8-2 and

in the second stage in Table 8-3) under the same temperature and photoperiod. This suggests that higher diapause intensity may be induced by diurnally variable field temperatures than by exposure to a constant temperature. Kalushkov *et al.* (2001) found this was the case in *Pyrrhocoris apterus* since a thermoperiod of 25/15°C evoked greater intensity of diapause than did a constant temperature of 25°C. However, the pre-oviposition period from collection to oviposition in the first stage (4.6 days) shown in Table 8-2 is shorter than that (13.9 days) shown in Table 8-1, but very close to that of the third stage (6.5 days, Table 8-2) and that of the second stage (6.6 days, Table 8-3). This may be due to the field-collected bugs considered in Table 8-2 having been kept temporarily at 15°C for some time (about 10 days) before they were exposed to 25°C/16L:8D. Temporary exposure at 15°C may have altered the diapause intensity of bugs, indicating that a period at a lower temperature is a prerequisite for diapause termination.

Ecological significance of diapause. In nearly all insects, diapause is a strictly seasonal adaptation within the life cycle. The adaptive value of diapause is in synchronising the occurrence of active stages with favourable seasons and in bringing about a complex of features increasing resistance to adverse climatic conditions (Hodek 1971). My work shows that in spring and summer when photoperiods are long, reproduction is rapid allowing insects to take maximum advantage of previously empty habitats. In late summer and autumn, the transition from long to short days induces diapause, and subsequently reproductive delay until the following spring. Diapause prevents reproductive activity in late summer and early autumn, despite the presence of suitable food and climatic conditions.

CHAPTER NINE

Survey of natural enemies of *N. huttoni*

Chapter 9

Survey of natural enemies of *N. huttoni*

9.1 Introduction

Biological control is an important part of integrated pest management (IPM) program. Biological control is the conscious use of beneficial living organisms, called natural enemies, to control pests attacking plants and animals. The natural enemies include parasitoids, predators, pathogens, antagonists, or competitor populations. Targets of biological control can be insects, mites, weeds, plant diseases, and vertebrates (Driesche & Bellows 1996). Biological control in an ecological sense is a population-level process in which the populations of natural enemies suppress pest population densities to levels lower than they would otherwise be by mechanisms such as predation, parasitism, pathogenicity, or competition. Natural enemies can regulate pest population because they act in a density-dependent manner (Debach & Rosen 1991, Driesche & Bellows 1996).

There have been three general approaches to biological control in the 20th century: (1) importation of new natural enemies and their establishment in a new habitat (classical biological control); (2) mass culture and periodic release of natural enemies (augmentation); and (3) conservation of existing natural enemies through manipulation of the environment (Pedigo 1989, Debach & Rosen 1991, Driesche & Bellows 1996, Luck *et al* 1999, Orr & Suh, 2000). Each of these methods can be used either alone or in combination in a biological control program.

Natural enemies play an important role in limiting potential pest populations. Successful biological control needs proper identification and better understanding of the organisms involved, both injurious and beneficial, and their intricate interaction. When successful, it is energy-efficient, cost-effective,

environmentally-safe, and compatible with other IPM tactics. Detailed knowledge of the systematics, biology and ecology of pests and their natural enemies is an essential foundation upon which the applications of biological control rest (Gullan & Cranston 2000, Debach & Rosen 1991).

The natural enemies of hemipteran pests in general include parasitic wasps, ants, cockroaches, dragonflies, some species of Reduviidae, Coreidae, Lygaeidae, Asilidae, and Strepsiptera, as well as some spiders, mites, mammals, birds, reptiles, and bats (Miller 1956). However, knowledge of the natural enemies of *N. huttoni* in particular is lacking. Therefore, one of the objects of my research was to look for possible predators as potential biological control agents.

9.2 Materials and methods

In the habitats where *N. huttoni* occurs year around, potential natural enemies were looked for between 1995 and 1999. The tiger beetle, *Neocicindela parryi*, the ground beetle, *Metaglymma moniliferum* Bates 1867, the ladybird beetle, *Coccinella trifasciata* L., the damsel bug, *Nabis maoricus*, and some species of spiders were seen near *N. huttoni*. In order to test whether or not these animals could or would eat *N. huttoni*, four species of insects, belonging to three families in two orders, and five species of spiders, belonging to four families in one order were collected and offered *N. huttoni* in the laboratory. Details of individual tests are given in Table 9 along with the results. Eggs, 3-5th instar nymphs, and adults of *N. huttoni* were given to predators. Fresh shepherd's purse was offered to *N. huttoni* as food each day.

9.3 Results

All nine predatory arthropods tested preyed on *N. huttoni* in the laboratory (Table 9).

Table 9. Results of feeding trials in which predatory insects and spiders were offered adults, nymphs or eggs of *N. huttoni* in the laboratory.

Predator	Stage of predator	Treatment (1 predator + no. prey)	Replicate	No. rearing days	Mean no. prey taken per predator per day		
					Egg	Nymph	Adult
Insects							
<i>Neocicindela parryi</i> (Coleoptera: Cicindelidae)	Adult	1. 1 + 10 adults	1	14	---	---	4.1 (1-8)
	Adult	2. 1 + 20 nymphs	2	4	---	8.3 (5-10)	---
	Adult*	3. 1 + 20 nymphs (1st stage)	1	11	---	9.9 (5-16)	---
		1 + 10 adults (2nd stage)	1	11	---	---	5.5 (0-10)
	Larva	4. 1 + 20 nymphs	1	6	---	2.5 (1-4)	---
<i>Metaglymma monilifer</i> (Coleoptera: Carabidae)	Adult	1 + 20 nymphs	1	6	---	8.0 (4-15)	---
<i>Coccinella trifasciata</i> L. (Coleoptera: Coccinellidae)	Adult	1. 1 + 20 eggs	35	3	14.3 (12-16)	---	---
	Adult	2. 1 + 5 nymphs	35	3	---	0.4 (0-2)	---
	Adult	3. 1 + 10 adults	35	3	---	---	0.0
<i>Nabis maoricus</i> (Hemiptera: Nabidae)	Adult*	1. 1 + 10 nymphs (1st stage)	1	16	---	1.6 (0-4)	---
		1 + 5 adults (2nd stage)	1	44	---	---	0.4 (0-2)
	Nymph	2. 1 + 10 nymphs	1	6	---	2.2 (0-5)	---
	Nymph	3. 1 + 5 adults	1	6	---	---	1.5 (0-3)
Spiders							
<i>Lycosa hilaris</i> Koch (Araneae: Lycosidae)	Adult	1. 1 + 5 nymphs	6	11.7 (4-33)	---	1.8 (0-5)	---
	Adult	2. 1 + 5 adults	3	18.7 (9-32)	---	---	0.4 (0-3)
<i>Pardosa bellicosa</i> (Goyen) (Araneae: Lycosidae)	Adult	1. 1 + 5 nymphs	8	5.3 (4-14)	---	2.3 (0-5)	---
	Adult	2. 1 + 5 adults	2	43 (33-53)	---	---	0.6 (0-5)
<i>Badumna longinqua</i> (Koch)** (Araneae: Desidae)	---	---	---	---	---	---	---
<i>Supunna picta</i> (Koch)** (Araneae: Corrinidae)	---	---	---	---	---	---	---
<i>Steatoda grossa</i> (Koch)** (Araneae: Theridiidae)	---	---	---	---	---	---	---

* The rearing period of the predator was divided into two stages. In the first stage the predator was fed on nymphs of *N. huttoni* and on adults in the second stage. ** No tests were conducted to determine the number of *N. huttoni* taken by each of these three species of spiders.

9.3.1 Insects

(a) *Neocicindela parryi*

Four tests were run with *N. parryi*. In the 1st test, one adult was kept for 14 days and offered 10 adults of *N. huttoni* each day. It ate on average 4.1 (range 1-8) adults per day. In test 2, two adults of *N. parryi* were kept, separately for four days and offered 20 nymphs of *N. huttoni* per day per predator. The tiger beetles consumed an average of 8.3 (5-10) nymphs per day. Thus, the number of nymphs consumed was twice the number of adults per day. In test 3, one adult of *N. parryi* was kept for 22 days. For the first 11 days, it was given 20 nymphs per day and it consumed an average of 9.9 (5-16) of them per day. For the second 11 days, it was offered 10 adults per day and the mean number consumed was 5.5 (0-10) per day, i.e., about half the adults were consumed. In the fourth test, a larva of *N. parryi* was kept for 6 days and was given 20 nymphs of *N. huttoni* daily. The mean number of prey eaten was 2.5 (1-4) per day, considerable fewer than were eaten by adult *N. parryi*.

When *N. parryi* attacked *N. huttoni* nymphs, it usually devoured the whole body of the prey. However, sometimes *N. parryi* merely sucked the body fluid from the prey and left the corpse complete but empty and flat. When *N. parryi* attacked *N. huttoni* adults, it ate most of the body but not the wings and legs. Sometimes, the bodies of *N. huttoni* adults were torn into fragments.

(b) *Metaglymma monilifer*

Only adults of *M. monilifer* were collected and tested. One adult, maintained for 6 days with 20 nymphs of *N. huttoni* offered daily, preyed upon a mean of 8 (4-15) nymphs per day, devouring the whole bodies. Another adult given 4 *N. huttoni* (2 nymphs and 2 adults) each day for two days ate them all. *M. monilifer* is only active at night, it is mainly a subsurface feeder, and its predation pressure on *N. huttoni* would be low.

(c) *Coccinella trifasciata*

C. trifasciata adults were offered 20 eggs, 5 nymphs, and 10 adults of *N. huttoni*, in each of three tests with 35 replicates. The tests were run for three days. In the first test, all 35 *C. trifasciata* adults fed on eggs during the three-day test and the mean predation rate was 14.3 (12-16) eggs/predator per day. This suggests *C. trifasciata* is an important predator of *N. huttoni* eggs. In the second test, 27 (77.1%) out of 35 *C. trifasciata* adults attacked *N. huttoni* nymphs within 3 days and each predator consumed an average of 0.4 (0-2) prey per day. In the third test, however, not one of 35 adults of *C. trifasciata* was observed to prey on adults of *N. huttoni* during the three-day test, possibly due to the bigger body size of the prey compared with that of the beetle.

When *C. trifasciata* adults fed on eggs, some ate the entire egg, but some ate only their contents, leaving the chorion. When adults attacked *N. huttoni* nymphs, most of them killed the nymphs and sucked the body fluids from them, with the corpse being left shrunk and flat. A few *C. trifasciata* adults devoured nymphs entirely or left only legs.

(d) *Nabis maoricus*

Three tests were run. In the first test, one *N. maoricus* adult was kept for 60 days. During the first 16 days, it was given 10 nymphs of *N. huttoni* per day and it attacked an average of 1.6 (0-4) prey per day. During the second 44 days, it was provided with 5 adults daily and it consumed 0.4 (0-2) prey per day. This suggests that *N. maoricus* adults can take more nymphs than adults of *N. huttoni*. In the second test, one *N. maoricus* nymph that was kept for 6 days and offered 10 nymphs daily, consumed an average of 2.2 (0-5) prey per day, while in the third test, one *N. maoricus* nymph that was also kept for 6 days, but was given 5 adults daily, attacked only 1.5 (0-3) prey per day. These two tests indicate that the nymph of *N. maoricus*, like the adult, has a higher capacity for killing nymphs than adults of *N. huttoni*.

Also, the predation rate of nymphs was higher than that of adults. One *N. maoricus* nymph attacked an average of 2.2 (0-5) nymphs (test 2) and 1.5 (0-3) adults (test 3) of *N. huttoni* per day, whereas, one *N. maoricus* adult captured an average of only 1.6 (0-4) nymphs or 0.4 (0-2) adults per day (test 1).

When *N. maoricus* adults and nymphs captured *N. huttoni*, they usually seized them with their fore legs and held them until the prey lost the ability to escape. The predator then started to search for a suitable position to insert the stylets into the body for sucking out the body fluid. Corpses were left flat and empty.

9.3.2 Spiders

(a) *Lycosa hilaris* Koch

Two tests were set up. In the first one, six replicates consisting of one adult *L. hilaris* provided with 5 nymphs of *N. huttoni* daily were established. The test replicates ran for an average of 11.7 (4-33) days. In the second test, one *L. hilaris* was given 5 adults daily (3 replicates) and ran for an average of 18.7 (9-32) days. The different length of times of replicates in the two tests was because spiders died on different days. Mean prey consumption by one adult *L. hilaris* was 1.8 nymphs per day (range 0-5), but only 0.4 adults were eaten (range 0-3)/spider per day.

Usually, the nymphs of *N. huttoni* were devoured or sucked empty by *L. hilaris* when they were attacked. Similarly, when *L. hilaris* attacked adults, most of the body was usually eaten, although indigestible, crushed cuticle and wings were dropped and left behind. Sometimes the prey was killed and torn into fragments without being eaten.

(b) *Pardosa bellicosa* (Goyen)

Two tests were run in which one *P. bellicosa* adult was offered either 5 nymphs or 5 adults. Eight trials with nymphal food were run for 4-14 days, and 2 trials with adults as food for 33-53 days. *P. bellicosa* adults captured an average of 2.3 nymphs (range 0-5) and 0.6 adults (range 0-5) daily.

P. bellicosa displayed a very similar feeding behaviour to *L. hilaris* when it attacked *N. huttoni*. Prey were usually devoured, but sometimes they were torn into fragments and eaten or not.

(c) *Badumna longinqua* (Koch), *Supunna picta* (Koch), and *Steatoda grossa* (Koch)

No specific feeding tests were conducted with these three species of spiders, but they were kept in the laboratory for about one month during which they were offered nymphs and adults of *N. huttoni*. My observations showed that all three species were able to capture *N. huttoni*. For example, one *S. picta* captured two 3rd-instar nymphs immediately they were provided. The first was eaten in two minutes, and the other was then attacked. Four more nymphs offered on the same day had all been eaten by the following day.

9.4 Summary and discussion

In the laboratory, an adult of *N. parryi* was able to consume 8-10 nymphs or 4-6 adults of *N. huttoni* per day, while a larva ate an average of only 2.5 *N. huttoni* nymphs. This indicates that *N. parryi* adults have a higher prey-killing capacity, at least in the laboratory. During the laboratory feeding test, adults of *M. monilifer* and *N. parryi* exhibited strong prey-killing capacity of *N. huttoni*. The daily predation rate on nymphs by *M. monilifer* was very similar to that exhibited by *N. parryi*, being about 8 prey/predator per day. In contrast to the above species of beetles, *C. trifasciata* preyed on large numbers of eggs of *N. huttoni* (mean 14.3 eggs/predator per day). As noted in Chapter 4, *N. huttoni*

lays eggs in the soil, and it is not known whether the ladybird beetles feed on eggs in the field.

Adults and nymphs of the fourth insect, *Nabis maoricus* preyed readily on nymphs and adults of *N. huttoni*, killing an average of 2.2 nymphs or 0.4 adults/predator per day in the absence of other prey. My results indicated that *N. maoricus* nymphs were more selective than the adults and preyed more readily on *N. huttoni* (both nymphs and adults). Clancy & Pierce (1966) reported similar findings for two other predatory nabids, *Nabis americanoferus* Carayon and *N. alternatus* Parshley and their prey, *Lygus hesperus*. However, adults of the tiger beetle, *N. parryi* preyed more heavily on *N. huttoni* than did their predatory larvae.

Lycosa hilaris and *Pardosa bellicosa* are wolf spiders, which were observed to dash between plants on a sunny summer's day. Forster & Forster (1999) stated that they are true hunting spiders and eat practically any invertebrate that is smaller than themselves. *Badumna longinqua*, the adventive Australian grey house spider, is the best known member of the Desidae in New Zealand and is now one of the commonest spiders in New Zealand (Forster & Forster 1999). A large numbers of *L. hilaris* and *P. bellicosa* were seen at my research site, but the density of *B. longinqua* was smaller. It is not known whether these spiders attacked *N. huttoni* in the field.

Supunna picta, commonly called the Australian ground spider, was also introduced to New Zealand from Australia. Since 1943 when it was found for the first time on Cuvier Island, *S. picta* has been reported to have expanded its distribution to most of the North Island, Marlborough, Nelson, West Coast, Canterbury, Central Otago, and Southland (Forster & Forster 1999). I collected *S. picta* at my research site in habitats similar to those described by Forster & Forster (1999). Spiders hunted in the sunshine, but it is not known whether they ate *N. huttoni* in the field.

Steatoda grossa, sometimes known as the house cobweb spider, was introduced from Europe and is one of 4 species of *Steatoda* in New Zealand. The two most common species, *S. grossa* and *S. capensis*, live mainly in and around houses, scrubland and the seashore (Forster & Forster 1999). I collected *S. grossa* only from the research site, but *S. capensis* was not found there. It is not known whether *S. capensis* preys on *N. huttoni* in the field.

Pseudatomoscelis seriatus (Reuter) (Nyffeler *et al.* 1992), *Lygus lineolaris* (Young & Lockley 1986), *Coptosoma variegata* Herich-Schaeffer, *Nezara viridula*, *Riptortus linearis*, and *Stephanitis typica* (Zhang shi-mei 1985) are hemipterans known to be attacked by spiders, while *Eurostus validus* Dallas, *Nezara viridula*, *Nysius ericae*, and *Stephanitis typica* are known to be attacked by ants. *Eteoneus angulatus* Drake et Maa and *Eurostus validus* are preyed on by Tettigoniidae species, and *Erthesina fullo*, *Homoeocerus walkerianus* Lethierry et Severin and *Riptortus linearis* by mantises. Dragonflies, lacewings (Chrysopidae), and *Orius* species are other known enemies of Hemiptera (Zhang shi-mei 1985). Reduviidae, the largest family of predaceous land Hemiptera, consists almost entirely of general feeders that also attack other species of Hemiptera both in the nymphal and adult stages (Ambrose 1999).

No parasitoids were found associated with *N. huttoni* although eggs of Hemiptera are liable to be attacked by various parasitoids of which the most important belong to the hymenopterous families, Scelionidae, Eupelmidae and Braconidae (Miller 1956). *Tessaratomia papillosa* is attacked by *Anastatus* sp., *Ooencyrtus corbetti*, *O. malayensis*, and *O. samargdina*; *Erthesina fullo* by *Telenomus* sp. and *Anostatus* sp.; *Nezara viridula* by *Telenomus gifuensis* and *Trissolcus* sp. (Zhang shi-mei 1985); *Lygus lineolaris* (Palisot) by *Peristebus digoneutis* (Day 1996); and *L. rugulipennis* Poppius by *Euphorus pallipes* (Curtis) (Stewart 1968). In my study, no parasitoids were found on *N. huttoni* eggs produced in the laboratory.

Eyles (1963c) reported that natural enemies of Lygaeidae include dipterous parasites, fungi and spiders, although he provided no information on natural enemies attacking *N. huttoni*. However, *Nysius vinitor* and *N. clevelandensis* were reported to be parasitised by *Alophora lepidofera* (Diptera: Tachinidae) in Australia, with average parasitisation rates of 16-62% for females and 0-5% for males (Attla 1973). *Nysius raphanus* and *N. ericae* are parasitised by *Hyalomya aldrichii* Townsend (Diptera: Tachinidae) in America (Clancy & Pierce 1966). A parasitic species of Tachinidae, *Trichopoda giacomellii* (Blanchard) is a biological control agent for *Nezara viridula* in Australia (Coombs & Sands 2000).

My laboratory study indicates that numerous arthropods may prey on the adults, nymphs and/or eggs of *N. huttoni* in the field although this has yet to be substantiated. The data obtained in this preliminary study should provide a useful foundation for further investigation.

CHAPTER TEN

Summary

Chapter 10

Summary

In this chapter, the findings of my research are summarised.

1. Habitats and host plants

Habitats and host plants of *N. huttoni* were investigated in the vicinity of Christchurch during 1995-1999. Forty-two host plants were found, 32 of which were recorded for the first time. *Spergularia rubra* and *Polygonum aviculare* were the most important spring and summer wild food plants, while *Erigeron karvinskianus* and *Lobularia maritima* were the most important cultivated food plants all year around. *N. huttoni* lives on the ground beneath plants and likes a dry, hot environment.

2. Temperature, photoperiod and development

Effects of temperature and photoperiod on development were studied in the laboratory. Hatching percentages of eggs, egg and nymphal development, mortality of nymphs, threshold temperature and thermal constant, number of instars, adult longevity and fecundity were determined at five constant temperatures (15, 20, 25, 30, and 35°C) under a photoperiod of 12L:12D and lab temperature (range 12.5-29.5°C, mean 20.3°C), as well as under three photoperiods (16-h, 12-h, and 8-h photophases) at 20 and 27.5°C. Shepherd's purse (*Capsella bursa-pastoris*) was used as food in all experimental procedures.

Hatching percentages of eggs. Hatching percentages of eggs were high and ranged from 79% to 91.2% under controlled temperatures and photoperiods. The highest hatching percentage of 95.5% was obtained at lab temperature.

Temperature and photoperiod had no apparent effect on hatching percentage ($P > 0.05$, One-way ANOVA).

Development of eggs and nymphs. Times for development of the egg stage, each nymphal instar, total nymphal stage (1-5 instars), and the total life cycle (egg to adult) were recorded under various laboratory conditions. A linear relationship was found between temperature and rate of development. Regression equations are given. These equations fit the observed values very well, as indicated by high values of the coefficients of determination (r^2). Mean duration of the egg stage at constant temperatures of 15, 20, 25, 30, and 35°C was 24.2, 13.3, 5.1, 5.1, and 3.3 days, respectively, and it was 5.9 days at lab temperature. Nymphal development required an average of 84.7 (15°C), 44.5 (20°C), 22.5 (25°C), 18.1 (30°C), and 14.5 days (35°C), respectively. Nymphs completed development more rapidly (38.8 days) at lab temperature than at a constant temperature (20°C) equal to the mean lab temperature. At all temperatures, second instars required least time for development and fifth instars required most time. Instar development for both sexes was similar at each temperature ($P > 0.05$, T-test).

Threshold temperature and thermal constant. Developmental thresholds and thermal requirements for the egg stage, each instar, total nymphal stage, and total life cycle were determined in the laboratory at five temperatures. Threshold temperature for egg development was 13.3°C, for nymphs 11.1°C, and for total development (egg to adult) 11.5°C. Threshold temperatures for different instars differed slightly. Thermal unit requirements for egg development, nymphal stage, and total development were 71.8, 341.3, and 419.1 day-degrees, respectively. Lower temperature limits for development of eggs and nymphs were similar (11°C). However, the upper temperature limit for egg development (45.2°C) was higher than that for nymphal development (41.7°C).

Photoperiod and development. Photoperiod affected development of *N. huttoni*. Development durations of eggs and nymphs were significantly shorter under a 12-h photoperiod than under 16- and 8-h photoperiods at 20°C. At 27.5°C, however, no difference in developmental time (egg to adult) was detected between 16-h and 12-h photoperiods, but development time under these two photoperiods was shorter than under the 8-h photoperiod ($P < 0.01$, One-way ANOVA).

Mortality of nymphs. Nymphs exhibited higher mortality in early than late instars, at low temperature than high temperature, and under a short photoperiod (8-h photophase) than mid-long photoperiods (12-h and 16-h photophases). Highest mortality of nymphs occurred in the 1st instar. Of all the nymphs that died, most died in the first instar.

Number of instars. In the laboratory, over 90% of the nymphs completed 5 instars and became adults. However, of the adults produced in laboratory populations, 2.8-10.9% had 4 nymphal instars and 1.2-5.2% had 6 nymphal instars. Their production and frequency of occurrence were related to temperature and photoperiod. Low temperature was favourable for producing more 4-instar adults and high temperature for producing more 6-instar adults. Short photoperiod (8L:16D) induced both 4 and 6-instar adults. Intermediate (about 25°C) and/or fluctuating temperature was optimal for producing normal 5-instar individuals. Developmental durations of 4- and 6-instar adults were not significantly different from those of 5-instar adults in most cases.

Adult longevity. Mean adult longevity at constant temperatures of 15, 20, 25, 30, and 35°C was 196.2, 119, 57, 16.9, and 8.6 days, respectively. No significant difference in the longevity of males and females was found in the range 15-30°C ($P > 0.05$, T-test). However, adult females lived longer than males at $\geq 35^\circ\text{C}$ (11.3 vs 8.6 days, $P < 0.01$). Over the temperature range tested, adult longevity decreased linearly as temperature increased. The

average adult life span at fluctuating lab temperature was 114.9 days (males: 113.7 days; females: 116.1 days) and was not significantly different from the mean longevity of 119 days (males: 120.7 days; females: 117.3 days) ($P > 0.05$, T-test) at 20°C. Under 16-h, 12-h, and 8-h photoperiods, adult longevity, which was determined only at 20°C, was 80.3, 120.1, and 159.5 days, respectively. Adults lived significantly longer under an 8-h than a 16-h photoperiod ($P < 0.05$, One-way ANOVA), but no differences were detected between the 16-h and 12-h, and the 12-h and 8-h photoperiods ($P > 0.05$).

Egg production. Total egg production per female (292.9 eggs/♀), relative and absolute oviposition periods (99.8 and 70.9 days), and number of days of successive oviposition (21.4 days) were highest at 20°C. Females produced up to 484 eggs in a life time (20°C). The highest relative and absolute numbers of eggs laid per female per day were obtained at 35°C (8.8 and 9 eggs/♀) and the lowest were at 15°C (0.6 and 2.1 eggs/♀). A female could lay up to 22 eggs a day (35°C). The lengths of pre- and post-oviposition periods were inversely related to temperature. The former ranged from 155.8 days at 15°C to 1.5 days at 35°C and the latter from 20.3 days at 15°C to 1.1 days at 35°C. Total number of eggs per female, absolute oviposition period, and daily egg production per female were substantially lower under an 8-h photophase than under 16-h and 12-h photophases ($P < 0.05$, One-way ANOVA).

3. Life history, biology and ecology

Detailed studies on aspects of the biology and life history of *N. huttoni* were carried out in the field near Christchurch in 1995-1999.

Overwintering. *N. huttoni* overwintered as adults of the third generation in mid-April - early May, and emerged from hibernation in late August or early September. Overwintering and emergence times were mainly determined by

temperature. Females laid eggs in soil, but no eggs were found in or on host plants.

Number of generations and occurrence. The number of generations per year was determined in two ways; by direct field observations at 5-day intervals during 1995-1999, and by sampling at 10-day intervals during 1995-1996 and 1996-1997. Both approaches indicated that three overlapping generations occurred each year in Canterbury. Information on developmental duration, the period of occurrence of each generation and the life stages in the field was obtained. Insects completed development of the first generation (egg to adult) in about 55-65 days, the second generation in 35-50 days, and the third generation in 20-60 days. The overall periods of occurrence for the three generations were 120-135, 80-120, and 285-295 days, respectively. The occurrence period of the third-generation adults (225-270 days) consisted of three stages: before winter (55-75 days), over winter (105-145 days), and after winter (60-65 days). Adults of the first (60-70 days) and second (45-70 days) generations were found for shorter periods.

Seasonal variation in population density at the field site was determined in 1995-1996 and 1996-1997. *N. huttoni* had a distinct seasonal pattern in abundance with three peaks in numbers, representing the three generations, each year.

Copulation and oviposition activities. Seasonal copulation activities in the field were recorded at 5-day intervals over four years (1995-1999) and oviposition activities in the field were determined by dissections of females collected at 10-day intervals for two years (1997-1998 and 1998-1999). Three copulation peaks and three oviposition peaks were found each year. They were produced by the overwintered (third), first-, and second-generation adults, respectively. Copulation and oviposition of the third generation occurred after winter.

Sex ratio. The sex ratio of *N. huttoni* ($\text{♀}:\text{♂} = 1:x$) was determined from (1) adults that emerged in the laboratory from nymphs collected from the field in 1997-1998, (2) laboratory-reared adults kept under different temperatures and photoperiods, and (3) field-collected adults in 1995-1996. Sex ratios of field-collected and laboratory-emerged adults and the population at different temperatures were not significantly different from the expected 1:1 ratio ($P > 0.05$, χ^2 test). Sex ratios of adults reared under 8L:16D at 20°C and 12L:12D at 27.5°C were male-biased (1:1.86, $P < 0.05$ and 1:1.73, $P < 0.01$, respectively). In the field-collected population, the sex ratio was 1:1 at most times of the year and males outnumbered females in only a few samples.

A single copulation fertilising a female for life. Laboratory tests indicated that a single copulation could not fertilise a female for life. Sperm were able to maintain vitality for one to three months. Hatching percentages of fertilised eggs resulting from multiple copulations were higher than those resulting from a single copulation. No parthenogenesis was found.

4. Reproductive diapause

Adults of third-generation *N. huttoni* underwent reproductive diapause from late summer to spring in the vicinity of Christchurch. This was confirmed by (1) field observations – no fourth-generation nymphs appeared before winter, (2) dissections of female adults – the proportion of females with mature or developing eggs decreased after February, and (3) artificial breaking of reproductive diapause by high temperature and long daylight – all diapaused adults collected in the field broke diapause and laid eggs when transferred to 25°C/16L:8D conditions in March.

In the field, diapause was induced by the shortening of the days and the onset of cooler temperatures. Photoperiod may play the more important role in inducing and terminating diapause, whereas temperature probably modifies

the effect of photoperiod. Naturally-induced diapause of field-collected bugs was broken within about two weeks when they were transferred to 25°C/16L:8D conditions in the laboratory. In the field, reproductive diapause was also terminated within about two weeks in early spring. In contrast to field-collected bugs, females entered diapause within about one month at 20°C/8L:16D, and terminated diapause within about one week when transferred to 25°C/16L:8D. The critical photoperiod for reproductive diapause was estimated to be between 14 and 12.5 hours. Diapause intensity induced under variable conditions of temperature/photoperiod in the field was greater than that induced under conditions of constant temperature/photoperiod in the laboratory.

5. Behaviour

Nymphs and adults of both sexes of *N. huttoni* cannibalised eggs in the laboratory, and both nymphs and adults could eat 4-9 eggs in 24 hours. The presence or absence of plant food had no influence on the number of bugs that fed on eggs, or the number of eggs eaten in 24 hours. However, this behaviour was related to temperature and soil humidity.

Positive phototaxis was also observed in the laboratory, but it was not very strong in the field. Nymphs but not adults feigned death when disturbed. During copulation in an end-to-end position, the female dragged the male backwards, moving all the time. The reason for this seems to be that females are generally larger than males. Nymphs and adults also showed aggregation behaviour.

6. Starvation-longevity

Starvation-longevity of *N. huttoni* adults from each of the three generations was tested in the laboratory. Adults were derived from fifth instar nymphs

collected in the field during the peak occurrence of each generation. Mean starvation-longevity of first-generation adults (4.2 days) was about twice that of second- and third-generation adults (2.2-2.5 days), suggesting that starvation-longevity was affected by the nutritional condition of food available in the field during the nymphal stage. When water was offered to adults, starvation-longevity increased significantly, to an average of 8.6 days. When adults were provided with glucose in concentrations of 2-40%, life span increased, substantially. Maximum longevity (mean 49-51.6 days) was obtained at 10-20% concentrations of glucose, but was lower at higher (25-40%) and lower (2-15%) concentrations. Adults could survive on glucose alone, but could not reproduce at any concentration of glucose, presumably due to the absence of protein.

7. Wing polymorphism and flight

Proportion of three wing forms. Both sexes of *N. huttoni* occurred in three wing forms: macropterous (M), sub-brachypterous (Sb), and brachypterous (B). Numbers and percentages of each wing form were investigated for three years (1995-1998) during which 14,245 adults in 56 samples collected from the field population at 10-day intervals were examined. In the field, the M-form was predominant (94.1%), whereas the Sb- and B-forms comprised 5.5% and 0.4% of the population, respectively. The sex ratio (♀:♂) was 1:1.13 in the M-form ($P < 0.01$, χ^2 test), 1:0.73 ($P < 0.01$) in the Sb-form, and 1:0.87 ($P > 0.05$) in the B-form.

Proportion of copulating combinations. Numbers and percentages of copulating pairs among the three wing forms were investigated for two years (1995-1997). Of the nine theoretical mating combinations, only 6 (M x M, ♀M x ♂Sb, ♂M x ♀Sb, Sb x Sb, ♂M x ♀B, and ♂Sb x ♀B) were found. M x M was predominant, reaching 80.9%, and ♂M x ♀Sb was the second common combination at 13.7%.

Factors affecting wing length. Environmental factors affecting wing length were investigated in the laboratory. Temperature and photoperiod both affected development of wings. Low ($\leq 15^{\circ}\text{C}$) and high temperatures ($\geq 30^{\circ}\text{C}$) and short daylength (8-h photoperiod) at lower temperature tended to favour the production of Sb- and B-forms. Production of the B-form was also associated with a reduction in the number of nymphal instars.

Flight. Flight of *N. huttoni* was investigated in relation to wing form. Flight was performed mainly by the M-form; where Sb-forms rarely flew and the B-form was flightless. Temperature was a major factor initiating flight. When air temperature rose to 30°C in the field, and/or the ground temperature reached 40°C , flight took place in both sexes ($\text{♀}:\text{♂} = 1:1$). Furthermore, flight activity increased as temperature increased. Thus, flights were restricted to day time, most occurring between 12:00-3:00 pm. Relationships between age, starvation, sexual deprivation, light and flight were examined with the aim of providing a better understanding of the causal factors of displacement flights in *N. huttoni*. Mature (gravid) females did not fly. Flight of adults was enhanced by mate shortage and starvation, and influenced by light. Some individuals never flew, indicating there seems to be a behavioural polymorphism with two types of individuals, “flyers” and “non-flyers”.

8. Natural enemies

Searches were made for natural enemies of *N. huttoni* over 4 years (1995 to 1999). No parasitoids were found, but potential predators collected from the field were tested in the laboratory. They comprised four species of insects belonging to 4 families in two orders and five species of spiders belonging to 4 families in one order. The feeding tests showed that all nine of these predatory insects and spiders would attack *N. huttoni* under laboratory conditions, although it is unknown whether they feed on *N. huttoni* in the field.

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